(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 October 2001 (04.10.2001)

PCT

(10) Internati nal Publication Number
WO 01/72957 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/IB01/00664

(22) International Filing Date: 2 April 2001 (02.04.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/540,118 31 March 2000 (31.03.2000) US

(71) Applicant and
(72) Inventor: ITOH, Nobuyuki [JP/JP]; Yanagawa, 1-24-7, Otsu, Shiga 520-0014 (JP).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/72957 A2

(54) Title: FIBROBLAST GROWTH FACTOR-LIKE MOLECULES AND USES THEREOF

(57) Abstract: Novel FGF-like polypeptides and nucleic acid molecules encoding the same. The invention also provides vectors, host cells, selective binding agents, and methods for producing FGF-like polypeptides. Also provided for are methods for the treatment, diagnosis, amelioration, or prevention of diseases with FGF-like polypeptides.

FIBROBLAST GROWTH FACTOR-LIKE MOLECULES AND USES THEREOF

This application is a continuation-in-part application of U.S. Patent Application Serial No. 09/540,118, which was filed March 31, 2000, which is incorporated by reference herein in its entirety for any purpose.

Field of the Invention

The present invention relates to novel Fibroblast Growth Factor (FGF)-like polypeptides and nucleic acid molecules encoding the same. The invention also relates to vectors, host cells, pharmaceutical compositions, selective binding agents and methods for producing FGF-like polypeptides. Also provided for are methods for the diagnosis, treatment, amelioration, and/or prevention of diseases associated with FGF-like polypeptides.

Background of the Invention

Technical advances in the identification, cloning, expression and manipulation of nucleic acid molecules and the deciphering of the human genome have greatly accelerated the discovery of novel therapeutics. Rapid nucleic acid sequencing techniques can now generate sequence information at unprecedented rates and, coupled with computational analyses, allow the assembly of overlapping sequences into partial and entire genomes and the identification of polypeptide-encoding regions. A comparison of a predicted amino acid sequence against a database compilation of known amino acid sequences allows one to determine the extent of homology to previously identified sequences and/or structural landmarks. The cloning and expression of a polypeptide-encoding region of a nucleic acid molecule provides a polypeptide product

for structural and functional analyses. The manipulation of nucleic acid molecules and encoded polypeptides may confer advantageous properties on a product for use as a therapeutic.

In spite of the significant technical advances in genome research over the past decade, the potential for the development of novel therapeutics based on the human genome is still largely unrealized. Many genes encoding potentially beneficial polypeptide therapeutics, or those encoding polypeptides, which may act as "targets" for therapeutic molecules, have still not been identified.

Accordingly, it is an object of the invention to identify novel polypeptides and nucleic acid molecules encoding the same, which have diagnostic or therapeutic benefit.

Summary of the Invention

The present invention relates to novel FGF-like nucleic acid molecules and encoded polypeptides.

Typical members of the FGF family have a conserved region of approximately 120 amino acids, sometimes referred to as the core region. Among FGF family members the amino acid identity within this core region is approximately 30-70%. The core region of the novel FGF-like polypeptide of the instant invention is located approximately at amino acid residues 48-166 of SEQ ID NO: 2. The amino acid sequences for representative members of the FGF family are shown in Figure 3.

The invention provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from:

(a) the nucleotide sequence as set forth in SEQ ID NO: 1;

(b) a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NO: 3;

(c) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of (a) or (b), wherein the encoded polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3; and

(d) a nucleotide sequence complementary to any of (a)-(c).

The invention also provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from:

(a) a nucleotide sequence encoding a polypeptide that is at least about 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the polypeptide as set forth in SEQ ID NO: 3, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(b) a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence as set forth in SEQ ID NO: 1, wherein the encoded polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(c) a nucleotide sequence of SEQ ID NO: 1, (a), or (b) encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(d) a nucleotide sequence of SEQ ID NO: 1, or (a)-(d) comprising a fragment of at least about 16 nucleotides;

(e) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(d), wherein the polypeptide has

an activity of the polypeptide as set forth in SEQ ID NO: 3; and

(f) a nucleotide sequence complementary to any of (a)-(d).

The invention further provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from:

(a) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 3 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(b) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 3 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(c) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 3 with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(d) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 3 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(e) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 3 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(f) a nucleotide sequence of (a)-(e) comprising a fragment of at least about 16 nucleotides;

(g) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(f), wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3; and

(h) a nucleotide sequence complementary to any of (a)-(e).

The invention also provides for an isolated polypeptide comprising the amino acid sequence selected from:

(a) the mature amino acid sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 comprising a mature amino terminus at threonine residue number 23, and optionally further comprising an amino-terminal methionine;

(b) an amino acid sequence for an ortholog of SEQ ID NO: 3, wherein the encoded polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(c) an amino acid sequence that is at least about 70, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the amino acid sequence of SEQ ID NO: 3, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(d) a fragment of the amino acid sequence set forth in SEQ ID NO: 2 comprising at least about 25 amino acid residues, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(e) an amino acid sequence for an allelic variant or splice variant of either the amino acid sequence as set forth in SEQ ID NO: 2, or at least one of (a)-(c) wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3.

The invention further provides for an isolated

polypeptide comprising the amino acid sequence selected from:

(a) the amino acid sequence as set forth in SEQ ID NO: 3 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(b) the amino acid sequence as set forth in SEQ ID NO: 3 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(c) the amino acid sequence as set forth in SEQ ID NO: 3 with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(d) the amino acid sequence as set forth in SEQ ID NO: 2 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3; and

(e) the amino acid sequence as set forth in SEQ ID NO: 3, with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3.

Also provided are fusion polypeptides comprising the amino acid sequences of (a)-(e) above.

The present invention also provides for an expression vector comprising the isolated nucleic acid molecules as set forth herein, recombinant host cells comprising recombinant nucleic acid molecules as set forth herein, and a method of producing an FGF-like

polypeptide comprising culturing the host cells and optionally isolating the polypeptide so produced.

A transgenic non-human animal comprising a nucleic acid molecule encoding an FGF-like polypeptide is also encompassed by the invention. The FGF-like nucleic acid molecules are introduced into the animal in a manner that allows expression and increased levels of the FGF-like polypeptide, which may include increased circulating levels. The transgenic non-human animal is preferably a mammal.

Also provided are derivatives of the FGF-like polypeptides of the present invention.

Additionally provided are selective binding agents such as antibodies and peptides capable of specifically binding the FGF-like polypeptides of the invention. Such antibodies and peptides may be agonistic or antagonistic.

Pharmaceutical compositions comprising the nucleotides, polypeptides, or selective binding agents of the present invention and one or more pharmaceutically acceptable formulation agents are also encompassed by the invention. The pharmaceutical compositions are used to provide therapeutically effective amounts of the nucleotides or polypeptides of the present invention. The invention is also directed to methods of using the polypeptides, nucleic acid molecules, and selective binding agents.

The FGF-like polypeptides and nucleic acid molecules of the present invention may be used to treat, prevent, ameliorate, and/or detect diseases and disorders, including those recited herein.

The present invention also provides a method of assaying test molecules to identify a test molecule that binds to an FGF-like polypeptide. The method comprises

contacting an FGF-like polypeptide with a test molecule and determining the extent of binding of the test molecule to the polypeptide. The method further comprises determining whether such test molecules are agonists or antagonists of an FGF-like polypeptide. The present invention further provides a method of testing the impact of molecules on the expression of FGF-like polypeptide or on the activity of FGF-like polypeptide.

Methods of regulating expression and modulating (*i.e.*, increasing or decreasing) levels of an FGF-like polypeptide are also encompassed by the invention. One method comprises administering to an animal a nucleic acid molecule encoding an FGF-like polypeptide. In another method, a nucleic acid molecule comprising elements that regulate or modulate the expression of an FGF-like polypeptide may be administered. Examples of these methods include gene therapy, cell therapy, and anti-sense therapy as further described herein.

In another aspect of the present invention, the FGF-like polypeptides may be used for identifying receptors thereof ("FGF-like receptors"). Various forms of "expression cloning" have been extensively used for cloning receptors for protein ligands. See for example, H. Simonsen and H.F. Lodish, Trends in Pharmacological Sciences, vol. 15, 437-441 (1994), and Tartaglia *et al.*, *Cell*, 83:1263-1271 (1995). The isolation of the FGF-like receptor(s) is useful for identifying or developing novel agonists and antagonists of the FGF-like polypeptide-signaling pathway. Such agonists and antagonists include soluble FGF-like receptor(s), anti-FGF-like receptor selective binding agents (such as antibodies and derivatives thereof), small molecules, and antisense oligonucleotides, any of which can be used for treating one or more of the diseases or disorders, including those recited herein.

Brief Description of the Figures

Figure 1 depicts a nucleic acid sequence (SEQ ID NO:1) encoding the human FGF-like polypeptide. Also depicted is the amino acid sequence of the mature human FGF-like polypeptide (SEQ ID NO: 3) and its precursor (SEQ ID NO: 2).

Figure 2 depicts a nucleic acid sequence (SEQ ID NO:31) encoding the mouse ortholog of FGF-like polypeptide. Also depicted is the predicted amino acid sequence of the mature mouse ortholog of FGF-like polypeptide (SEQ ID NO: 33) and its precursor (SEQ ID NO: 32).

Figure 3 depicts the amino acid sequences from representative members of the FGF family (SEQ ID NO:4 - SEQ ID NO: 24).

Detailed Description of the Invention

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All references cited in this application are expressly incorporated by reference herein for any purpose.

Definitions

The terms "FGF-like gene" or "FGF-like nucleic acid molecule" or "polynucleotide" refers to a nucleic acid molecule comprising or consisting of a nucleotide sequence as set forth in SEQ ID NO: 1, a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NO: 3, and nucleic acid molecules as defined herein.

The term "FGF-like polypeptide" refers to a polypeptide comprising the amino acid sequence of SEQ ID NO: 3, and related polypeptides. Related polypeptides include: FGF-like polypeptide allelic variants, FGF-like polypeptide orthologs, FGF-like polypeptide splice variants, FGF-like polypeptide variants and FGF-like polypeptide derivatives. FGF-like polypeptides may be mature polypeptides, as defined herein, or precursor polypeptides, and may or may not have an amino terminal methionine residue, depending on the method by which they are prepared.

The term "FGF-like polypeptide allelic variant" refers to one of several possible naturally occurring alternate forms of a gene occupying a given locus on a chromosome of an organism or a population of organisms.

The term "FGF-like polypeptide derivatives" refers to the polypeptide as set forth in SEQ ID NO: 3, FGF-like polypeptide allelic variants, FGF-like polypeptide orthologs, FGF-like polypeptide splice variants, or FGF-like polypeptide variants, as defined herein, that have been chemically modified.

The term "FGF-like polypeptide fragment" refers to a polypeptide that comprises a truncation at the amino terminus (with or without a leader sequence) and/or a truncation at the carboxy terminus of the polypeptide as set forth in SEQ ID NO: 3, FGF-like polypeptide allelic variants, FGF-like polypeptide orthologs, FGF-like polypeptide splice variants and/or an FGF-like polypeptide variant having one or more amino acid additions or substitutions or internal deletions (wherein the resulting polypeptide is at least 6 amino acids or more in length) as compared to the FGF-like polypeptide amino acid sequence set forth in SEQ ID NO: 2. FGF-like

polypeptide fragments may result from alternative RNA splicing or from *in vivo* protease activity.

In preferred embodiments, truncations comprise about 10 amino acids, or about 20 amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or more than about 100 amino acids. The polypeptide fragments so produced will comprise about 25 contiguous amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or about 150 amino acids. Such FGF-like polypeptide fragments may optionally comprise an amino terminal methionine residue. It will be appreciated that such fragments can be used, for example, to generate antibodies to FGF-like polypeptides.

The term "FGF-like fusion polypeptide" refers to a fusion of one or more amino acids (such as a heterologous peptide or polypeptide) at the amino or carboxy terminus of the polypeptide as set forth in SEQ ID NO: 2 or SEQ ID NO: 3, FGF-like polypeptide allelic variants, FGF-like polypeptide orthologs, FGF-like polypeptide splice variants, or FGF-like polypeptide variants having one or more amino acid deletions, substitutions or internal additions as compared to the FGF-like polypeptide amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 3.

The term "FGF-like polypeptide ortholog" refers to a polypeptide from another species that corresponds to the FGF-like polypeptide amino acid sequence as set forth in SEQ ID NO: 2. For example, mouse and human FGF-like polypeptides are considered orthologs of each other.

The term "FGF-like polypeptide splice variant" refers to a nucleic acid molecule, usually RNA, which is generated by alternative processing of intron sequences

in an RNA transcript of FGF-like polypeptide amino acid sequence as set forth in SEQ ID NO: 2.

The term "FGF-like polypeptide variants" refers to FGF-like polypeptides comprising amino acid sequences having one or more amino acid sequence substitutions, deletions (such as internal deletions and/or FGF-like polypeptide fragments), and/or additions (such as internal additions and/or FGF-like fusion polypeptides) as compared to the FGF-like polypeptide amino acid sequence set forth in SEQ ID NO: 2 (with or without the leader sequence). Variants may be naturally occurring (*e.g.*, FGF-like polypeptide allelic variants, FGF-like polypeptide orthologs and FGF-like polypeptide splice variants) or artificially constructed. Such FGF-like polypeptide variants may be prepared from the corresponding nucleic acid molecules having a DNA sequence that varies accordingly from the DNA sequence as set forth in SEQ ID NO: 1. In preferred embodiments, the variants have from 1 to 3, or from 1 to 5, or from 1 to 10, or from 1 to 15, or from 1 to 20, or from 1 to 25, or from 1 to 50, or from 1 to 75, or from 1 to 100, or more than 100 amino acid substitutions, insertions, additions and/or deletions, wherein the substitutions may be conservative, or non-conservative, or any combination thereof.

The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody, and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes.

The term "biologically active FGF-like polypeptides" refers to FGF-like polypeptides having at least one

activity characteristic of the polypeptide comprising the amino acid sequence of SEQ ID NO: 3.

The terms "effective amount" and "therapeutically effective amount" each refer to the amount of a FGF-like polypeptide or FGF-like nucleic acid molecule used to support an observable level of one or more biological activities of the FGF-like polypeptides as set forth herein.

The term "expression vector" refers to a vector which is suitable for use in a host cell and contains nucleic acid sequences which direct and/or control the expression of heterologous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present.

The term "host cell" is used to refer to a cell which has been transformed, or is capable of being transformed with a nucleic acid sequence and then of expressing a selected gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present.

The term "identity" as known in the art, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between nucleic acid molecules or polypeptides, as the case may be, as determined by the match between strings of two or more nucleotide or two or more amino acid sequences. "Identity" measures the percent of identical matches between the smaller of two

or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (*i.e.*, "algorithms").

The term "similarity" is a related concept, but in contrast to "identity", refers to a measure of similarity which includes both identical matches and conservative substitution matches. If two polypeptide sequences have, for example, 10/20 identical amino acids, and the remainder are all non-conservative substitutions, then the percent identity and similarity would both be 50%. If in the same example, there are 5 more positions where there are conservative substitutions, then the percent identity remains 50%, but the per cent similarity would be 75% (15/20). Therefore, in cases where there are conservative substitutions, the degree of similarity between two polypeptides will be higher than the percent identity between those two polypeptides.

The term "isolated nucleic acid molecule" refers to a nucleic acid molecule of the invention that (1) has been separated from at least about 50 percent of proteins, lipids, carbohydrates or other materials with which it is naturally found when total DNA is isolated from the source cells, (2) is not linked to all or a portion of a polynucleotide to which the "isolated nucleic acid molecule" is linked in nature, (3) is operably linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature as part of a larger polynucleotide sequence. Preferably, the isolated nucleic acid molecule of the present invention is substantially free from any other contaminating nucleic acid molecule(s) or other contaminants that are found in its natural environment that would interfere with its use in polypeptide production or its therapeutic, diagnostic, prophylactic

or research use.

The term "isolated polypeptide" refers to a polypeptide of the present invention that (1) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates or other materials with which it is naturally found when isolated from the source cell, (2) is not linked (by covalent or noncovalent interaction) to all or a portion of a polypeptide to which the "isolated polypeptide" is linked in nature, (3) is operably linked (by covalent or noncovalent interaction) to a polypeptide with which it is not linked in nature, or (4) does not occur in nature. Preferably, the isolated polypeptide is substantially free from any other contaminating polypeptides or other contaminants that are found in its natural environment that would interfere with its therapeutic, diagnostic, prophylactic or research use.

The term "mature FGF-like polypeptide" refers to an FGF-like polypeptide lacking the leader sequence. A mature FGF-like polypeptide may also include other modifications such as proteolytic processing of the amino terminus (with or without a leader sequence) and/or the carboxy terminus, cleavage of a smaller polypeptide from a larger precursor, N-linked and/or O-linked glycosylation, and the like. An exemplary mature FGF-like polypeptide is depicted by SEQ ID NO: 3 or by amino acid residue 23 through amino acid residue 170 of SEQ ID NO:2.

The term "nucleic acid sequence" or "nucleic acid molecule" refers to a DNA or RNA sequence. The term encompasses molecules formed from any of the known base analogs of DNA and RNA such as, but not limited to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinyl-cytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl)

uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethyl-guanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyamino-methyl-2-thiouracil, beta-D-mannosylqueosine, 5' -methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

The term "naturally occurring" or "native" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by man. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man.

The term "operably linked" is used herein to refer to an arrangement of flanking sequences wherein the flanking sequences so described are configured or assembled so as to perform their usual function. Thus, a flanking sequence operably linked to a coding sequence may be capable of effecting the replication, transcription and/or translation of the coding sequence. For example, a coding sequence is operably linked to a promoter when the promoter is capable of directing

transcription of that coding sequence. A flanking sequence need not be contiguous with the coding sequence, so long as it functions correctly. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

The term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to one or more formulation materials suitable for accomplishing or enhancing the delivery of the FGF-like polypeptide, FGF-like nucleic acid molecule or FGF-like selective binding agent as a pharmaceutical composition.

The term "selective binding agent" refers to a molecule or molecules having specificity for an FGF-LIKE polypeptide. As used herein, the terms, "specific" and "specificity" refer to the ability of the selective binding agents to bind to human FGF-like polypeptides and not to bind to human non-FGF-like polypeptides. It will be appreciated, however, that the selective binding agents may also bind orthologs of the polypeptide as set forth in SEQ ID NO: 3, that is, interspecies versions thereof, such as mouse and rat polypeptides.

The term "transduction" is used to refer to the transfer of genes from one bacterium to another, usually by a phage. "Transduction" also refers to the acquisition and transfer of eukaryotic cellular sequences by retroviruses.

The term "transfection" is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are

disclosed herein. See, for example, Graham *et al.*, *Virology*, 52:456 (1973); Sambrook *et al.*, *Molecular Cloning, a laboratory Manual*, Cold Spring Harbor Laboratories (New York, 1989); Davis *et al.*, *Basic Methods in Molecular Biology*, Elsevier, 1986; and Chu *et al.*, *Gene*, 13:197 (1981). Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

The term "transformation" as used herein refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection or transduction, the transforming DNA may recombine with that of the cell by physically integrating into a chromosome of the cell, may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to have been stably transformed when the DNA is replicated with the division of the cell.

The term "vector" is used to refer to any molecule (*e.g.*, nucleic acid, plasmid, or virus) used to transfer coding information to a host cell.

Relatedness of Nucleic Acid Molecules and/or Polypeptides

It is understood that related nucleic acid molecules include allelic or splice variants of the nucleic acid molecule of SEQ ID NO:1, and include sequences which are complementary to any of the above nucleotide sequences. Related nucleic acid molecules also include a nucleotide sequence encoding a polypeptide comprising or consisting essentially of a substitution, modification, addition

and/or a deletion of one or more amino acid residues compared to the polypeptide in SEQ ID NO: 3.

Fragments include molecules which encode a polypeptide of at least about 25 amino acid residues, or about 50, or about 75, or about 100, or greater than about 100 amino acid residues of the polypeptide of SEQ ID NO: 2.

In addition, related FGF-like nucleic acid molecules include those molecules which comprise nucleotide sequences which hybridize under moderately or highly stringent conditions as defined herein with the fully complementary sequence of the nucleic acid molecule of SEQ ID NO: 1, or of a molecule encoding a polypeptide, which polypeptide comprises the amino acid sequence as shown in SEQ ID NO: 3, or of a nucleic acid fragment as defined herein, or of a nucleic acid fragment encoding a polypeptide as defined herein. Hybridization probes may be prepared using the FGF-like sequences provided herein to screen cDNA, genomic or synthetic DNA libraries for related sequences. Regions of the DNA and/or amino acid sequence of FGF-like polypeptide that exhibit significant identity to known sequences are readily determined using sequence alignment algorithms as described herein and those regions may be used to design probes for screening.

The term "highly stringent conditions" refers to those conditions that are designed to permit hybridization of DNA strands whose sequences are highly complementary, and to exclude hybridization of significantly mismatched DNAs. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of "highly stringent conditions" for hybridization and washing are 0.015M sodium chloride,

0.0015M sodium citrate at 65-68°C or 0.015M sodium chloride, 0.0015M sodium citrate, and 50% formamide at 42°C. See Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, (Cold Spring Harbor, N.Y. 1989); Anderson *et al.*, Nucleic Acid Hybridisation: a practical approach, Ch. 4, IRL Press Limited (Oxford, England).

More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agent) may also be used, however, the rate of hybridization will be affected. Other agents may be included in the hybridization and washing buffers for the purpose of reducing non-specific and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinyl-pyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate ($NaDodSO_4$ or SDS), ficoll, Denhardt's solution, sonicated salmon sperm DNA (or other non-complementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these additives can be changed without substantially affecting the stringency of the hybridization conditions. Hybridization experiments are usually carried out at pH 6.8-7.4, however, at typical ionic strength conditions, the rate of hybridization is nearly independent of pH. See Anderson *et al.*, Nucleic Acid Hybridisation: a Practical Approach, Ch. 4, IRL Press Limited (Oxford, England).

Factors affecting the stability of a DNA duplex include base composition, length, and degree of base pair mismatch. Hybridization conditions can be adjusted by one skilled in the art in order to accommodate these variables and allow DNAs of different sequence relatedness to form hybrids. The melting temperature of a perfectly matched DNA duplex can be estimated by the

following equation:

$T_m(°C) = 81.5 + 16.6(\log[Na+]) + 0.41(\%G+C) - 600/N - 0.72(\%formamide)$

where N is the length of the duplex formed, [Na+] is the molar concentration of the sodium ion in the hybridization or washing solution, %G+C is the percentage of (guanine+cytosine) bases in the hybrid. For imperfectly matched hybrids, the melting temperature is reduced by approximately 1°C for each 1% mismatch.

The term "moderately stringent conditions" refers to conditions under which a DNA duplex with a greater degree of base pair mismatching than could occur under "highly stringent conditions" is able to form. Examples of typical "moderately stringent conditions" are 0.015M sodium chloride, 0.0015M sodium citrate at 50-65°C or 0.015M sodium chloride, 0.0015M sodium citrate, and 20% formamide at 37-50°C. By way of example, a "moderately stringent" condition of 50°C in 0.015 M sodium ion will allow about a 21% mismatch.

It will be appreciated by those skilled in the art that there is no absolute distinction between "highly" and "moderately" stringent conditions. For example, at 0.015M sodium ion (no formamide), the melting temperature of perfectly matched long DNA is about 71°C. With a wash at 65°C (at the same ionic strength), this would allow for approximately a 6% mismatch. To capture more distantly related sequences, one skilled in the art can simply lower the temperature or raise the ionic strength.

A good estimate of the melting temperature in 1M NaCl* for oligonucleotide probes up to about 20nt is given by:

Tm = 2°C per A-T base pair + 4°C per G-C base pair

*The sodium ion concentration in 6X salt sodium citrate (SSC) is 1M. See Suggs *et al.*, Developmental Biology Using Purified Genes, p. 683, Brown and Fox (eds.) (1981).

High stringency washing conditions for oligonucleotides are usually at a temperature of 0-5°C below the Tm of the oligonucleotide in 6X SSC, 0.1% SDS.

In another embodiment, related nucleic acid molecules comprise or consist of a nucleotide sequence that is about 70 percent identical to the nucleotide sequence as shown in SEQ ID NO: 1, or comprise or consist essentially of a nucleotide sequence encoding a polypeptide that is about 70 percent identical to the polypeptide as set forth in SEQ ID NO: 3. In preferred embodiments, the nucleotide sequences are about 75 percent, or about 80 percent, or about 85 percent, or about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the nucleotide sequence as shown in SEQ ID NO: 1, or the nucleotide sequences encode a polypeptide that is about 75 percent, or about 80 percent, or about 85 percent, or about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the polypeptide sequence as set forth in SEQ ID NO: 3.

Differences in the nucleic acid sequence may result in conservative and/or non-conservative modifications of the amino acid sequence relative to the amino acid sequence of SEQ ID NO: 3.

Conservative modifications to the amino acid sequence of SEQ ID NO: 2 (and the corresponding modifications to the encoding nucleotides) will produce FGF-like polypeptides having functional and chemical characteristics similar to those of naturally occurring FGF-like polypeptide. In contrast, substantial

modifications in the functional and/or chemical characteristics of FGF-like polypeptides may be accomplished by selecting substitutions in the amino acid sequence of SEQ ID NO: 2 that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis."

Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted forms of amino acid moieties.

Naturally occurring residues may be divided into classes based on common side chain properties:

1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
3) acidic: Asp, Glu;
4) basic: His, Lys, Arg;
5) residues that influence chain orientation: Gly, Pro; and
6) aromatic: Trp, Tyr, Phe.

For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the human FGF-like polypeptide that are homologous with non-human FGF-like polypeptide orthologs, or into the non-homologous regions of the molecule.

In making such changes, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art. Kyte *et al.*, *J. Mol. Biol.*, 157:105-131 (1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the

biologically functionally equivalent protein or peptide thereby created is intended for use in immunological embodiments, as in the present case. The greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.*, with a biological property of the protein.

The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the FGF-like polypeptide, or to increase or decrease the affinity of the FGF-like polypeptides described herein.

Exemplary amino acid substitutions are set forth in Table I.

Table I

Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth in SEQ ID NO: 2 using well known techniques. For identifying suitable areas of the molecule that may be changed without destroying activity, one skilled in the art may target areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of an FGF-like polypeptide to such similar polypeptides. With such a comparison, one can identify residues and portions of the molecules that are conserved among similar polypeptides. It will be appreciated that changes in areas of an FGF-like polypeptide that are not conserved relative to such similar polypeptides would be less likely to adversely affect the biological activity and/or structure of the FGF-like polypeptide. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in an FGF-like polypeptide that correspond to amino acid residues that are important for activity or structure in similar polypeptides. One skilled in the art may opt for chemically similar amino acid substitutions for such

predicted important amino acid residues of FGF-like polypeptides.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of that information, one skilled in the art may predict the alignment of amino acid residues of an FGF-like polypeptide with respect to its three dimensional structure. One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moult J., *Curr. Op. in Biotech.*, 7(4):422-427 (1996), Chou *et al.*, *Biochemistry*, 13(2):222-245 (1974); Chou *et al.*, *Biochemistry*, 113(2):211-222 (1974); Chou *et al.*, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 47:45-148 (1978); Chou *et al.*, *Ann. Rev. Biochem.*, 47:251-276 and Chou *et al.*, *Biophys. J.*, 26:367-384 (1979). Moreover, computer

programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural data base (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. *See* Holm *et al.*, *Nucl. Acid. Res.*, 27(1):244-247 (1999). It has been suggested (Brenner *et al.*, *Curr. Op. Struct. Biol.*, 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will gain dramatically in accuracy.

Additional methods of predicting secondary structure include "threading" (Jones, D., Curr. Opin. Struct. Biol., 7(3):377-87 (1997); Sippl et al., Structure, 4(1):15-9 (1996)), "profile analysis" (Bowie et al., Science, 253:164-170 (1991); Gribskov et al., Meth. Enzym., 183:146-159 (1990); Gribskov et al., Proc. Nat. Acad. Sci., 84(13):4355-4358 (1987)), and "evolutionary linkage" (See Home, supra, and Brenner, supra).

Preferred FGF-like polypeptide variants include glycosylation variants wherein the number and/or type of glycosylation sites has been altered compared to the amino acid sequence set forth in SEQ ID NO: 3. In one embodiment, FGF-like polypeptide variants comprise a greater or a lesser number of N-linked glycosylation sites than the amino acid sequence set forth in SEQ ID NO: 3. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the

amino acid residue designated as X may be any amino acid residue except proline. The substitution(s) of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions which eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. Additional preferred FGF-like variants include cysteine variants, wherein one or more cysteine residues are deleted from or substituted for another amino acid (e.g., serine) as compared to the amino acid sequence set forth in SEQ ID NO: 3. Cysteine variants are useful when FGF-like polypeptides must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

In addition, the polypeptide comprising the amino acid sequence of SEQ ID NO: 3 or an FGF-like polypeptide variant may be fused to a homologous polypeptide to form a homodimer or to a heterologous polypeptide to form a heterodimer. Heterologous peptides and polypeptides include, but are not limited to: an epitope to allow for the detection and/or isolation of an FGF-like fusion polypeptide; a transmembrane receptor protein or a portion thereof, such as an extracellular domain, or a transmembrane and intracellular domain; a ligand or a portion thereof which binds to a transmembrane receptor protein; an enzyme or portion thereof which is catalytically active; a polypeptide or peptide which

promotes oligomerization, such as a leucine zipper domain; a polypeptide or peptide which increases stability, such as an immunoglobulin constant region; and a polypeptide which has a therapeutic activity different from the polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 3 or an FGF-like polypeptide variant.

Fusions can be made either at the amino terminus or at the carboxy terminus of the polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 3 or an FGF-like polypeptide variant. Fusions may be direct with no linker or adapter molecule or indirect using a linker or adapter molecule. A linker or adapter molecule may be one or more amino acid residues, typically up to about 20 to about 50 amino acid residues. A linker or adapter molecule may also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties. It will be appreciated that once constructed, the fusion polypeptides can be derivatized according to the methods described herein.

In a further embodiment of the invention, the polypeptide comprising the amino acid sequence of SEQ ID NO: 3 or an FGF-like polypeptide variant is fused to one or more domains of an Fc region of human IgG. Antibodies comprise two functionally independent parts, a variable domain known as "Fab", which binds antigen, and a constant domain known as "Fc", which is involved in effector functions such as complement activation and attack by phagocytic cells. An Fc has a long serum half-life, whereas an Fab is short-lived. Capon *et al.*, *Nature*, 337:525-31 (1989). When constructed together with a therapeutic protein, an Fc domain can provide longer half-life or incorporate such functions as Fc

receptor binding, protein A binding, complement fixation and perhaps even placental transfer. *Id.* Table II summarizes the use of certain Fc fusions known in the art.

Table II
Fc Fusion with Therapeutic Proteins

Form of Fc	Fusion partner	Therapeutic implications	Reference
IgG1	N-terminus of CD30-L	Hodgkin's disease; anaplastic lymphoma; T-cell leukemia	U.S. Patent No. 5,480,981
Murine Fcγ2a	IL-10	anti-inflammatory; transplant rejection	Zheng *et al.* (1995), *J. Immunol.*, 154: 5590-5600
IgG1	TNF receptor	septic shock	Fisher *et al.* (1996), *N. Engl. J. Med.*, 334: 1697-1702; Van Zee *et al.*, (1996), *J. Immunol.*, 156: 2221-2230
IgG, IgA, IgM, or IgE (excluding the first domain)	TNF receptor	inflammation, autoimmune disorders	U.S. Pat. No. 5,808,029, issued September 15, 1998
IgG1	CD4 receptor	AIDS	Capon *et al.* (1989), *Nature* 337: 525-531
IgG1, IgG3	N-terminus of IL-2	anti-cancer, antiviral	Harvill *et al.* (1995), *Immunotech.*, 1: 95-105
IgG1	C-terminus of OPG	osteoarthritis; bone density	WO 97/23614, published July 3, 1997
IgG1	N-terminus of leptin	anti-obesity	PCT/US 97/23183, filed December 11, 1997
Human Ig Cγ1	CTLA-4	autoimmune disorders	Linsley (1991), *J. Exp. Med.*, 174:561-569

In one example, all or a portion of the human IgG hinge, CH2 and CH3 regions may be fused at either the N-

terminus or C-terminus of the FGF-like polypeptides using methods known to the skilled artisan. The resulting FGF-like fusion polypeptide may be purified by use of a Protein A affinity column. Peptides and proteins fused to an Fc region have been found to exhibit a substantially greater half-life *in vivo* than the unfused counterpart. Also, a fusion to an Fc region allows for dimerization/multimerization of the fusion polypeptide. The Fc region may be a naturally occurring Fc region, or may be altered to improve certain qualities, such as therapeutic qualities, circulation time, reduce aggregation, etc.

Identity and similarity of related nucleic acid molecules and polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo *et al.*, *SIAM J. Applied Math.*, 48:1073 (1988).

Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are described in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux *et al.*, *Nucl. Acid. Res.*, 12:387

(1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Altschul *et al.*, *J. Mol. Biol.*, 215:403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (*BLAST Manual*, Altschul *et al.* NCB/NLM/NIH Bethesda, MD 20894; Altschul *et al.*, *supra*). The well known Smith Waterman algorithm may also be used to determine identity.

Certain alignment schemes for aligning two amino acid sequences may result in the matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full length sequences. Accordingly, in a preferred embodiment, the selected alignment method (GAP program) will result in an alignment that spans at least 50 contiguous amino acids of the target polypeptide.

For example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, WI), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated as 3X the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. A standard comparison matrix (see Dayhoff *et al.*, Atlas of Protein Sequence and Structure, vol. 5, supp.3 (1978) for th PAM 250

comparison matrix; Henikoff *et al.*, *Proc. Natl. Acad. Sci* USA, 89:10915-10919 (1992) for the BLOSUM 62 comparison matrix) is also used by the algorithm.

Preferred parameters for a polypeptide sequence comparison include the following:

Algorithm: Needleman *et al.*, *J. Mol. Biol.*, 48:443-453 (1970);
Comparison matrix: BLOSUM 62 from Henikoff *et al.*, *Proc. Natl. Acad. Sci.* USA, 89:10915-10919 (1992);
Gap Penalty: 12
Gap Length Penalty: 4
Threshold of Similarity: 0

The GAP program is useful with the above parameters. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

Preferred parameters for nucleic acid molecule sequence comparisons include the following:

Algorithm: Needleman *et al.*, *J. Mol Biol.*, 48:443-453 (1970);
Comparison matrix: matches = +10, mismatch = 0
Gap Penalty: 50
Gap Length Penalty: 3

The GAP program is also useful with the above parameters. The aforementioned parameters are the default parameters for nucleic acid molecule comparisons.

Other exemplary algorithms, gap opening penalties, gap extension penalties, comparison matrices, thresholds of similarity, etc. may be used,, including those set

forth in the Program Manual, Wisconsin Package, Version 9, September, 1997. The particular choices to be made will be apparent to those of skill in the art and will depend on the specific comparison to be made, such as DNA to DNA, protein to protein, protein to DNA; and additionally, whether the comparison is between given pairs of sequences (in which case GAP or BestFit are generally preferred) or between one sequence and a large database of sequences (in which case FASTA or BLASTA are preferred).

Synthesis

It will be appreciated by those skilled in the art the nucleic acid and polypeptide molecules described herein may be produced by recombinant and other means.

Nucleic Acid Molecules

The nucleic acid molecules encode a polypeptide comprising the amino acid sequence of an FGF-like polypeptide can readily be obtained in a variety of ways including, without limitation, chemical synthesis, cDNA or genomic library screening, expression library screening and/or PCR amplification of cDNA.

Recombinant DNA methods used herein can be those set forth in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), and/or Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, Green Publishers Inc. and Wiley and Sons, NY (1994). The present invention provides for nucleic acid molecules as described herein and methods for obtaining the molecules.

Where a gene encoding the amino acid sequence of an FGF-like polypeptide has been identified from one

species, all or a portion of that gene may be used as a probe to identify orthologs or related genes from the same species. The probes or primers may be used to screen cDNA libraries from various tissue sources believed to express the FGF-like polypeptide. In addition, part or all of a nucleic acid molecule having the sequence as set forth in SEQ ID NO: 1 may be used to screen a genomic library to identify and isolate a gene encoding the amino acid sequence of an FGF-like polypeptide. Typically, conditions of moderate or high stringency will be employed for screening to minimize the number of false positives obtained from the screen.

Nucleic acid molecules encoding the amino acid sequence of FGF-like polypeptides may also be identified by expression cloning which employs the detection of positive clones based upon a property of the expressed protein. Typically, nucleic acid libraries are screened by the binding of an antibody or other binding partner (*e.g.*, receptor or ligand) to cloned proteins which are expressed and displayed on a host cell surface. The antibody or binding partner is modified with a detectable label to identify those cells expressing the desired clone.

Recombinant expression techniques conducted in accordance with the descriptions set forth below may be followed to produce these polynucleotides and to express the encoded polypeptides. For example, by inserting a nucleic acid sequence which encodes the amino acid sequence of an FGF-like polypeptide into an appropriate vector, one skilled in the art can readily produce large quantities of the desired nucleotide sequence. The sequences can then be used to generate detection probes or amplification primers. Alternatively, a polynucleotide encoding the amino acid sequence of an

FGF-like polypeptide can be inserted into an expression vector. By introducing the expression vector into an appropriate host, the encoded FGF-like polypeptide may be produced in large amounts.

Another method for obtaining a suitable nucleic acid sequence is the polymerase chain reaction (PCR). In this method, cDNA is prepared from poly(A)+RNA or total RNA using the enzyme reverse transcriptase. Two primers, typically complementary to two separate regions of cDNA (oligonucleotides) encoding the amino acid sequence of an FGF-like polypeptide, are then added to the cDNA along with a polymerase such as *Taq* polymerase, and the polymerase amplifies the cDNA region between the two primers.

Another means of preparing a nucleic acid molecule encoding the amino acid sequence of an FGF-like polypeptide is chemical synthesis using methods well known to the skilled artisan such as those described by Engels *et al.*, *Angew. Chem. Intl. Ed.*, 28:716-734 (1989). These methods include, *inter alia*, the phosphotriester, phosphoramidite, and H-phosphonate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA encoding the amino acid sequence of an FGF-like polypeptide will be several hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form the full length nucleotide sequence of an FGF-like polypeptide. Usually, the DNA fragment encoding the amino terminus of the polypeptide will have an ATG, which encodes a methionine residue. This methionine may or may not be present on the mature form of the FGF-like polypeptide, depending on

whether the polypeptide produced in the host cell is designed to be secreted from that cell. Other methods known to the skilled artisan may be used as well.

In certain embodiments, nucleic acid variants contain codons which have been altered for the optimal expression of an FGF-like polypeptide in a given host cell. Particular codon alterations will depend upon the FGF-like polypeptide(s) and host cell(s) selected for expression. Such "codon optimization" can be carried out by a variety of methods, for example, by selecting codons which are preferred for use in highly expressed genes in a given host cell. Computer algorithms which incorporate codon frequency tables such as "Ecohigh.cod" for codon preference of highly expressed bacterial genes may be used and are provided by the University of Wisconsin Package Version 9.0, Genetics Computer Group, Madison, WI. Other useful codon frequency tables include "Celegans_high.cod", "Celegans_low.cod", "Drosophila_high.cod", "Human_high.cod", "Maize_high.cod", and "Yeast_high.cod".

Vectors and Host Cells

A nucleic acid molecule encoding the amino acid sequence of an FGF-like polypeptide may be inserted into an appropriate expression vector using standard ligation techniques. The vector is typically selected to be functional in the particular host cell employed (*i.e.*, the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can occur). A nucleic acid molecule encoding the amino acid sequence of an FGF-like polypeptide may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems), and/or eukaryotic host cells. Selection of the host cell will depend in part on whether an FGF-like polypeptide is to be post-translationally modified (*e.g.*, glycosylated and/or phosphorylated). If

so, yeast, insect, or mammalian host cells are preferable. For a review of expression vectors, see *Meth. Enz.*, v.185, D.V. Goeddel, ed. Academic Press Inc., San Diego, CA (1990).

Typically, expression vectors used in any of the host cells will contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as "flanking sequences" in certain embodiments will typically include one or more of the following nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these sequences is discussed below.

Optionally, the vector may contain a "tag"-encoding sequence, *i.e.*, an oligonucleotide molecule located at the 5' or 3' end of the FGF-like polypeptide coding sequence; the oligonucleotide sequence encodes polyHis (such as hexaHis), or other "tag" such as FLAG, HA (hemaglutinin Influenza virus) or *myc* for which commercially available antibodies exist. This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification of the FGF-like polypeptide from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified FGF-like polypeptide by various means such as using certain peptidases for cleavage.

Flanking sequences may be homologous (*i.e.*, from the same species and/or strain as the host cell), heterologous (*i.e.*, from a species other than the host cell species or strain), hybrid (*i.e.*, a combination of flanking sequences from more than one source) or synthetic, or the flanking sequences may be native sequences which normally function to regulate FGF-like polypeptide expression. As such, the source of a flanking sequence may be any prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking sequence is functional in, and can be activated by, the host cell machinery.

The flanking sequences useful in the vectors of this invention may be obtained by any of several methods well known in the art. Typically, flanking sequences useful herein other than the FGF-like gene flanking sequences will have been previously identified by mapping and/or by restriction endonuclease digestion and can thus be isolated from the proper tissue source using the appropriate restriction endonucleases. In some cases, the full nucleotide sequence of a flanking sequence may be known. Here, the flanking sequence may be synthesized using the methods described herein for nucleic acid synthesis or cloning.

Where all or only a portion of the flanking sequence is known, it may be obtained using PCR and/or by screening a genomic library with suitable oligonucleotide and/or flanking sequence fragments from the same or another species. Where the flanking sequence is not known, a fragment of DNA containing a flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion to produce the proper DNA fragment followed by isolation using agarose gel

purification, Qiagen® column chromatography (Chatsworth, CA), or other methods known to the skilled artisan. The selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

An origin of replication is typically a part of those prokaryotic expression vectors purchased commercially, and the origin aids in the amplification of the vector in a host cell. Amplification of the vector to a certain copy number can, in some cases, be important for the optimal expression of an FGF-like polypeptide. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector. For example, the origin of replication from the plasmid pBR322 (Product No. 303-3s, New England Biolabs, Beverly, MA) is suitable for most Gram-negative bacteria and various origins (*e.g.*, SV40, polyoma, adenovirus, vesicular stomatitus virus (VSV) or papillomaviruses such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (for example, the SV40 origin is often used only because it contains the early promoter).

A transcription termination sequence is typically located 3' of the end of a polypeptide coding region and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment followed by a poly T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using methods for nucleic acid synthesis such as those described herein.

A selectable marker gene element encodes a protein necessary for the survival and growth of a host cell

grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, tetracycline, or kanamycin for prokaryotic host cells, (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex media. According to certain embodiments, preferred selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. A neomycin resistance gene may also be used for selection in prokaryotic and eukaryotic host cells.

Other selection genes may be used to amplify the gene which will be expressed. Amplification is the process wherein genes which are in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Examples of suitable selectable markers for mammalian cells in certain embodiments include dihydrofolate reductase (DHFR) and thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of the selection gene present in the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to the amplification of both the selection gene and the DNA that encodes an FGF-like polypeptide. As a result, increased quantities of FGF-like polypeptide are synthesized from the amplified DNA.

A ribosome binding site is typically used for translation initiation of mRNA and is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically located 3' to the

promoter and 5' to the coding sequence of an FGF-like polypeptide to be expressed. The Shine-Dalgarno sequence is varied but is typically a polypurine (*i.e.*, having a high A-G content). Many Shine-Dalgarno sequences have been identified, each of which can be made using methods set forth herein and used in a prokaryotic vector.

In certain embodiments, a leader, or signal, sequence may be used to direct an FGF-like polypeptide out of the host cell. Typically, a nucleotide sequence encoding the signal sequence is positioned in the coding region of an FGF-like nucleic acid molecule, or directly at the 5' end of an FGF-like polypeptide coding region. Many signal sequences have been identified, and a signal sequence that is functional in the selected host cell may be used in conjunction with an FGF-like nucleic acid molecule. A signal sequence may be homologous (naturally occurring) or heterologous to an FGF-like gene or cDNA. Additionally, a signal sequence may be chemically synthesized using methods described herein. In most cases, the secretion of an FGF-like polypeptide from the host cell via the presence of a signal peptide will result in the removal of the signal peptide from the secreted FGF-like polypeptide. The signal sequence may be a component of the vector, or it may be a part of an FGF-like nucleic acid molecule that is inserted into the vector.

Included within the scope of this invention is the use of either a nucleotide sequence encoding a native FGF-like polypeptide signal sequence joined to an FGF-like polypeptide coding region or a nucleotide sequence encoding a heterologous signal sequence joined to an FGF-like polypeptide coding region. The heterologous signal sequence selected should be one that is recognized and processed, *i.e.*, cleaved by a signal peptidase, by the host cell. For prokaryotic host cells that do not recognize and process the native FGF-like polypeptide

signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, or heat-stable enterotoxin II leaders. For yeast secretion, the native FGF-like polypeptide signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various presequences to improve glycosylation or yield. For example, one may alter the peptidase cleavage site of a particular signal peptide, or add presequences, which also may affect glycosylation. The final protein product may have, in the -1 position (relative to the first amino acid of the mature protein) one or more additional amino acids incident to expression, which may not have been totally removed. For example, the final protein product may have one or two amino acid residues found in the peptidase cleavage site, attached to the N-terminus. Alternatively, use of some enzyme cleavage sites may result in a slightly truncated form of the desired FGF-like polypeptide, if the enzyme cuts at such area within the mature polypeptide.

In many cases, transcription of a nucleic acid molecule is increased by the presence of one or more introns in the vector; this is particularly true where a polypeptide is produced in eukaryotic host cells, especially mammalian host cells. The introns used may be naturally occurring within the FGF-like gene, especially where the gene used is a full length genomic sequence or a fragment thereof. Where the intron is not naturally occurring within the gene (as for most cDNAs), the intron(s) may be obtained from another source. The

position of the intron with respect to flanking sequences and the FGF-like gene is generally important, as the intron must be transcribed to be effective. Thus, when an FGF-like cDNA molecule is being transcribed, the preferred position for the intron is 3' to the transcription start site, and 5' to the polyA transcription termination sequence. Preferably, the intron or introns will be located on one side or the other (*i.e.*, 5' or 3') of the cDNA such that it does not interrupt the coding sequence. Any intron from any source, including any viral, prokaryotic and eukaryotic (plant or animal) organisms, may be used to practice this invention, provided that it is compatible with the host cell(s) into which it is inserted. Also included herein are synthetic introns. Optionally, more than one intron may be used in the vector.

The expression and cloning vectors of the present invention will each typically contain a promoter that is recognized by the host organism and operably linked to the molecule encoding a FGF-like polypeptide. Promoters are untranscribed sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription of the structural gene. Promoters are conventionally grouped into one of two classes, inducible promoters and constitutive promoters. Inducible promoters initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, such as the presence or absence of a nutrient or a change in temperature. Constitutive promoters, on the other hand, initiate continual gene product production; that is, there is little or no control over gene expression. A large number of promoters, recognized by a variety of potential host cells, are well known. A suitable promoter is operably linked to the DNA encoding an FGF-

like polypeptide by removing the promoter from the source DNA by restriction enzyme digestion and inserting the desired promoter sequence into the vector. The native FGF-like gene promoter sequence may be used to direct amplification and/or expression of an FGF-LIKE nucleic acid molecule. A heterologous promoter is preferred, however, if it permits greater transcription and higher yields of the expressed protein as compared to the native promoter, and if it is compatible with the host cell system that has been selected for use.

Promoters suitable for use with prokaryotic hosts include the beta-lactamase and lactose promoter systems; alkaline phosphatase, a tryptophan (trp) promoter system; and hybrid promoters such as the tac promoter. Other known bacterial promoters are also suitable. Their sequences have been published, thereby enabling one skilled in the art to ligate them to the desired DNA sequence(s), using linkers or adapters as needed to supply any useful restriction sites.

Suitable promoters for use with yeast hosts are also well known in the art. Yeast enhancers are advantageously used with yeast promoters. Suitable promoters for use with mammalian host cells are well known and include, but are not limited to, those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus (CMV), a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40). Other suitable mammalian promoters include heterologous mammalian promoters, *e.g.*, heat-shock promoters and the actin promoter.

Additional promoters which may be of interest in controlling FGF-like gene transcription include, but are

not limited to: the SV40 early promoter region (Bernoist and Chambon, *Nature*, 290:304-310, 1981); the CMV promoter; the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, *Cell*, 22:787-797, 1980); the herpes thymidine kinase promoter (Wagner *et al.*, *Proc. Natl. Acad. Sci. USA*, 78:144-1445, 1981); the regulatory sequences of the metallothionine gene (Brinster *et al.*, *Nature*, 296:39-42, 1982); prokaryotic expression vectors such as the beta-lactamase promoter (Villa-Kamaroff, *et al.*, *Proc. Natl. Acad. Sci. USA*, 75:3727-3731, 1978); or the tac promoter (DeBoer, *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:21-25, 1983). Also of interest are the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, *Cell*, 38:639-646, 1984; Ornitz *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, 50:399-409 (1986); MacDonald, *Hepatology*, 7:425-515, 1987); the insulin gene control region which is active in pancreatic beta cells (Hanahan, *Nature*, 315:115-122, 1985); the immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, *Cell*, 38:647-658 (1984); Adames *et al.*, *Nature*, 318:533-538 (1985); Alexander *et al.*, *Mol. Cell. Biol.*, 7:1436-1444, 1987); the mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, *Cell*, 45:485-495, 1986); the albumin gene control region which is active in liver (Pinkert *et al.*, *Genes and Devel.*, 1:268-276, 1987); the alphafetoprotein gene control region which is active in liver (Krumlauf *et al.*, *Mol. Cell. Biol.*, 5:1639-1648, 1985; Hammer *et al.*, *Science*, 235:53-58, 1987); the alpha 1-antitrypsin gene control region which is active in the liver (Kelsey *et al.*, *Genes and Devel.*, 1:161-171, 1987); the beta-globin

gene control region which is active in myeloid cells (Mogram *et al.*, *Nature*, 315:338-340, 1985; Kollias *et al.*, *Cell*, 46:89-94, 1986); the myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, *Cell*, 48:703-712, 1987); the myosin light chain-2 gene control region which is active in skeletal muscle (Sani, *Nature*, 314:283-286, 1985); and the gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, Science, 234:1372-1378, 1986).

An enhancer sequence may be inserted into the vector to increase the transcription of a DNA encoding an FGF-like polypeptide of the present invention by higher eukaryotes. Enhancers are cis-acting elements of DNA, usually about 10-300 bp in length, that act on the promoter to increase transcription. Enhancers are relatively orientation and position independent. They have been found 5' and 3' to the transcription unit. Several enhancer sequences available from mammalian genes are known (*e.g.*, globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus will be used. The SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer may be spliced into the vector at a position 5' or 3' to an FGF-like nucleic acid molecule, it is typically located at a site 5' from the promoter.

Expression vectors of the invention may be constructed from a starting vector such as a commercially available vector. Such vectors may or may not contain all of the desired flanking sequences. Where one or more of the desired flanking sequences are not already present

in the vector, they may be individually obtained and ligated into the vector. Methods used for obtaining each of the flanking sequences are well known to one skilled in the art.

Vectors that can be used for certain embodiments of the invention may be those which are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pcDNA3.1 (Invitrogen Company, Carlsbad, CA), pBSII (Stratagene Company, La Jolla, CA), pET15b (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech, Palo Alto, CA), pETL (BlueBacII; Invitrogen), pDSR-alpha (PCT Publication No. WO90/14363) and pFastBacDual (Gibco/BRL, Grand Island, NY).

Additional suitable vectors include, but are not limited to, cosmids, plasmids or modified viruses, but it will be appreciated that the vector system must be compatible with the selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript® plasmid derivatives (a high copy number ColE1-based phagemid, Stratagene Cloning Systems Inc., La Jolla CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products (*e.g.*, TOPO™ TA Cloning® Kit, PCR2.1® plasmid derivatives, Invitrogen, Carlsbad, CA), and mammalian, yeast, or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech, Palo Alto, CA).

After the vector has been constructed and a nucleic acid molecule encoding an FGF-like polypeptide has been inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or polypeptide expression. The transformation of an expression vector for an FGF-like polypeptide into a selected host cell may be

accomplished by well known methods including methods such as transfection, infection, calcium chloride, electroporation, microinjection, lipofection or the DEAE-dextran method or other known techniques. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, *supra*.

According to certain embodiments, host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a yeast cell, an insect cell or a vertebrate cell). According to certain embodiments, the host cell, when cultured under appropriate conditions, synthesizes an FGF-like polypeptide which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). According to certain embodiments, the selection of an appropriate host cell may depend upon various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity, such as glycosylation or phosphorylation, and ease of folding into a biologically active molecule.

A number of suitable host cells are known in the art and many are available from the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209. Examples include, but are not limited to, mammalian cells, such as Chinese hamster ovary cells (CHO) (ATCC No. CCL61) CHO DHFR-cells (Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA*, 97:4216-4220 (1980)), human embryonic kidney (HEK) 293 or 293T cells (ATCC No. CRL1573), or 3T3 cells (ATCC No. CCL92). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art.

Other suitable mammalian cell lines, are the monkey COS-1 (ATCC No. CRL1650) and COS-7 cell lines (ATCC No. CRL1651), and the CV-1 cell line (ATCC No. CCL70). Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, as well as primary explants, are also suitable. In certain embodiments, candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines, which are available from the ATCC. Each of these cell lines is known by and available to those skilled in the art of protein expression.

Similarly useful as host cells suitable for the present invention are bacterial cells. For example, the various strains of *E. coli* (*e.g.*, HB101, (ATCC No. 33694) DH5α, DH10, and MC1061 (ATCC No. 53338)) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas spp.*, other *Bacillus spp.*, *Streptomyces spp.*, and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for the expression of the polypeptides of the present invention. Preferred yeast cells include, for example, *Saccharomyces cerivisae* and *Pichia pastoris*.

Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described for example in Kitts *et al.*, *Biotechniques*, 14:810-817 (1993); Lucklow, *Curr. Opin.*

Biotechnol., 4:564-572 (1993); and Lucklow *et al.* (*J. Virol.*, 67:4566-4579 (1993). Preferred insect cells are Sf-9 and Hi5 (Invitrogen, Carlsbad, CA).

One may also use transgenic animals to express glycosylated FGF-like polypeptides. For example, one may use a transgenic milk-producing animal (a cow or goat, for example) and obtain the present glycosylated polypeptide in the animal milk. One may also use plants to produce FGF-like polypeptides, however, in general, the glycosylation occurring in plants is different from that produced in mammalian cells, and may result in a glycosylated product which is not suitable for human therapeutic use.

Polypeptide Production

Host cells comprising an FGF-like polypeptide expression vector may be cultured using standard media well known to the skilled artisan. In certain embodiments, the media contains all nutrients necessary for the growth and survival of the cells. Suitable media for culturing *E. coli* cells include, for example, Luria Broth (LB) and/or Terrific Broth (TB). Exemplary media for culturing eukaryotic cells include Roswell Park Memorial Institute medium 1640 (RPMI 1640), Minimal Essential Medium (MEM) and/or Dulbecco's Modified Eagle Medium (DMEM), all of which may be supplemented with serum and/or growth factors as indicated by the particular cell line being cultured. An exemplary medium for insect cultures is Grace's medium supplemented with yeastolate, lactalbumin hydrolysate and/or fetal calf serum, as necessary.

In certain embodiments, an antibiotic or other compound useful for selective growth of transformed cells

is added as a supplement to the media. The compound to be used may be dictated by the selectable marker element present on the plasmid with which the host cell was transformed. For example, where the selectable marker element is kanamycin resistance, the compound added to the culture medium will be kanamycin. Other compounds for selective growth include ampicillin, tetracycline, and neomycin.

In certain embodiments, the amount of an FGF-like polypeptide produced by a host cell can be evaluated using standard methods known in the art. Such methods include, without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, HPLC separation, immunoprecipitation, and/or activity assays such as DNA binding gel shift assays.

If an FGF-like polypeptide has been designed to be secreted from the host cells, the majority of polypeptide typically may be found in the cell culture medium. If however, the FGF-like polypeptide is not secreted from the host cells, it may be present in the cytoplasm and/or the nucleus (for eukaryotic host cells) or in the cytosol (for bacterial host cells).

For an FGF-like polypeptide situated in the host cell cytoplasm and/or the nucleus (for eukaryotic host cells) or in the cytosol (for bacterial host cells), intracellular material (including inclusion bodies for gram-negative bacteria) can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication followed by centrifugation.

If an FGF-like polypeptide has formed inclusion bodies in the cytosol, the inclusion bodies can often bind to the inner and/or outer cellular membranes and thus may be found primarily in the pellet material after centrifugation. The pellet material can then be treated at pH extremes or with a chaotropic agent such as a detergent, guanidine, guanidine derivatives, urea, or urea derivatives in the presence of a reducing agent such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. The FGF-like polypeptide in its now soluble form can then be analyzed using gel electrophoresis, immunoprecipitation or the like. If it is desired to isolate the FGF-like polypeptide, isolation may be accomplished using standard methods such as those described herein and in Marston *et al.*, *Meth. Enz.*, 182:264-275 (1990).

In some cases, an FGF-like polypeptide may not be biologically active upon isolation. Various methods for "refolding" or converting the polypeptide to its tertiary structure and generating disulfide linkages can be used to restore biological activity. Such methods include exposing the solubilized polypeptide to a pH typically above 7 and in the presence of a particular concentration of a chaotrope. The selection of chaotrope is very similar to the choices used for inclusion body solubilization, but typically the chaotrope is used at a lower concentration and is not necessarily the same as chaotropes used for the solubilization. In certain cases the refolding/oxidation solution will also contain a reducing agent or the reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential allowing for disulfide shuffling to occur in the formation of the protein's cysteine bridge(s). Some of the commonly used redox couples include

cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol(DTT)/ dithiane DTT, and 2-2mercaptoethanol(bME)/dithio-b(ME). A cosolvent may be used to increase the efficiency of the refolding, and the common reagents used for this purpose include glycerol, polyethylene glycol of various molecular weights, arginine and the like.

If inclusion bodies are not formed to a significant degree upon expression of an FGF-like polypeptide, then the polypeptide typically will be found primarily in the supernatant after centrifugation of the cell homogenate. The polypeptide may be further isolated from the supernatant using methods such as those described herein.

The purification of an FGF-like polypeptide from solution can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (FGF-like polypeptide/hexaHis) or other small peptide such as FLAG (Eastman Kodak Co., New Haven, CT) or *myc* (Invitrogen, Carlsbad, CA) at either its carboxyl or amino terminus, it may be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag.

For example, polyhistidine binds with great affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen® nickel columns) can be used for purification of FGF-like polypeptide/polyHis. See for example, Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, Section 10.11.8, John Wiley & Sons, New York (1993).

Additionally, the FGF-like polypeptide may be purified through the use of a monoclonal antibody which

is capable of specifically recognizing and binding to the FGF-like polypeptide.

Suitable procedures for purification thus include, without limitation, affinity chromatography, immunoaffinity chromatography, ion exchange chromatography, molecular sieve chromatography, High Performance Liquid Chromatography (HPLC), electrophoresis (including native gel electrophoresis) followed by gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific, San Francisco, CA). In some cases, two or more purification techniques may be combined to achieve increased purity.

FGF-like polypeptides may also be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using techniques known in the art, such as those set forth by Merrifield *et al.*, *J. Am. Chem. Soc.*, 85:2149 (1963), Houghten *et al.*, *Proc Natl Acad. Sci. USA*, 82:5132 (1985), and Stewart and Young, Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL (1984). Such polypeptides may be synthesized with or without a methionine on the amino terminus. Chemically synthesized FGF-like polypeptides may be oxidized using methods set forth in these references to form disulfide bridges. Chemically synthesized FGF-like polypeptides are expected to have comparable biological activity to the corresponding FGF-like polypeptides produced recombinantly or purified from natural sources, and thus may be used interchangeably with a recombinant or natural FGF-like polypeptide.

Another way to obtain an FGF-like polypeptide is via purification from biological samples such as source tissues and/or fluids in which the FGF-like polypeptide is naturally found. Such purification can be conducted using methods for protein purification as described

herein. The presence of the FGF-like polypeptide during purification may be monitored using, for example, an antibody prepared against recombinantly produced FGF-like polypeptide or peptide fragments thereof.

A number of additional methods for producing nucleic acids and polypeptides are known in the art, and can be used to produce polypeptides having specificity for FGF-like polypeptide. See for example, Roberts *et al.*, *Proc. Natl. Acad. Sci.*, 94:12297-12303 (1997), which describes the production of fusion proteins between an mRNA and its encoded peptide. *See also* Roberts, R., *Curr. Opin. Chem. Biol.*, 3:268-273 (1999). Additionally, U.S. Patent No. 5,824,469 describes methods of obtaining oligonucleotides capable of carrying out a specific biological function. The procedure involves generating a heterogeneous pool of oligonucleotides, each having a 5′ randomized sequence, a central preselected sequence, and a 3′ randomized sequence. The resulting heterogeneous pool is introduced into a population of cells that do not exhibit the desired biological function. Subpopulations of the cells are then screened for those which exhibit a predetermined biological function. From that subpopulation, oligonucleotides capable of carrying out the desired biological function are isolated.

U.S. Patent Nos. 5,763,192, 5,814,476, 5,723,323, and 5,817,483 describe processes for producing peptides or polypeptides. This is done by producing stochastic genes or fragments thereof, and then introducing these genes into host cells which produce one or more proteins encoded by the stochastic genes. The host cells are then screened to identify those clones producing peptides or polypeptides having the desired activity.

Chemical Derivatives

Chemically modified derivatives of the FGF-like polypeptides may be prepared by one skilled in the art, given the disclosures set forth hereinbelow. FGF-like polypeptide derivatives are modified in a manner that is different, either in the type or location of the molecules naturally attached to the polypeptide. Derivatives may include molecules formed by the deletion of one or more naturally-attached chemical groups. The polypeptide comprising the amino acid sequence of SEQ ID NO: 2, or an FGF-like polypeptide variant may be modified by the covalent attachment of one or more polymers. For example, in certain embodiments, the polymer selected is typically water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Included within the scope of suitable polymers is a mixture of polymers. In certain embodiments, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable.

The polymers each may be of any molecular weight and may be branched or unbranched. In certain embodiments, the polymers each typically have an average molecular weight of between about 2kDa to about 100kDa (the term "about" indicating that in preparations of a water soluble polymer, some molecules will weigh more, some less, than the stated molecular weight). In certain embodiments, the average molecular weight of each polymer is between about 5kDa and about 50kDa, in certain embodiments, between about 12kDa and about 40kDa, and in certain embodiments, between about 20kDa and about 35kDa.

Suitable water soluble polymers or mixtures thereof include, but are not limited to, N-linked or O-linked carbohydrates, sugars, phosphates, polyethylene glycol (PEG) (including the forms of PEG that have been used to

derivatize proteins, including mono-(C_1-C_{10}) alkoxy- or aryloxy-polyethylene glycol), monomethoxy-polyethylene glycol, dextran (such as low molecular weight dextran, of, for example about 6 kD), cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.*, glycerol) and polyvinyl alcohol. Also encompassed by certain embodiments of the present invention are bifunctional crosslinking molecules which may be used to prepare covalently attached multimers of the polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or an FGF-like polypeptide variant.

In certain embodiments, chemical derivatization may be performed under any suitable condition used to react a protein with an activated polymer molecule. Methods for preparing chemical derivatives of polypeptides in certain embodiments, comprise (a) reacting the polypeptide with the activated polymer molecule (such as a reactive ester or aldehyde derivative of the polymer molecule) under conditions whereby the polypeptide comprising the amino acid sequence of SEQ ID NO: 2, or an FGF-like polypeptide variant becomes attached to one or more polymer molecules, and (b) obtaining the reaction product(s). The optimal reaction conditions will be determined based on known parameters and the desired result. For example, the larger the ratio of polymer molecules:protein, the greater the percentage of attached polymer molecule. In certain embodiments, the FGF-like polypeptide derivative may have a single polymer molecule moiety at the amino terminus. See, for example, U.S. Patent No. 5,234,784.

Pegylation of a polypeptide specifically may be carried out by any of the pegylation reactions known in

the art, as described for example in the following references: Francis *et al.*, *Focus on Growth Factors*, 3:4-10 (1992); EP 0154316; EP 0401384 and U.S. Patent No. 4,179,337. For example, pegylation may be carried out via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule (or an analogous reactive water-soluble polymer) as described herein. For the acylation reactions, the polymer(s) selected typically should have a single reactive ester group. For reductive alkylation, the polymer(s) selected typically should have a single reactive aldehyde group. A reactive aldehyde is, for example, polyethylene glycol propionaldehyde, which is water stable, or mono C_1-C_{10} alkoxy or aryloxy derivatives thereof (see U.S. Patent No. 5,252,714).

In certain embodiments, FGF-like polypeptides may be chemically coupled to biotin, and the biotin/FGF-like polypeptide molecules which are conjugated are then allowed to bind to avidin, resulting in tetravalent avidin/biotin/FGF-like polypeptide molecules. FGF-like polypeptides may also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugates precipitated with anti-DNP or anti-TNP-IgM to form decameric conjugates with a valency of 10.

Typically, conditions which may be alleviated or modulated by the administration of the present FGF-like polypeptide derivatives include at least some of those described herein for FGF-like polypeptides. However, the FGF-like polypeptide derivatives disclosed herein may have additional activities, enhanced or reduced biological activity, or other characteristics, such as increased or decreased half-life, as compared to the non-derivatized molecules.

Genetically Engineered Non-Human Animals

Additionally included within the scope of the present invention are non-human animals such as mice, rats, or other rodents, rabbits, goats, or sheep, or other farm animals, in which the gene (or genes) encoding the native FGF-like polypeptide has (have) been disrupted ("knocked out") such that the level of expression of this gene or genes is (are) significantly decreased or completely abolished. Such animals may be prepared using techniques and methods such as those described in U.S. Patent No. 5,557,032.

The present invention further includes non-human animals such as mice, rats, or other rodents, rabbits, goats, sheep, or other farm animals, in which either the native form of the FGF-like gene(s) for that animal or a heterologous FGF-like gene(s) is (are) over-expressed by the animal, thereby creating a "transgenic" animal. Such transgenic animals may be prepared using well known methods such as those described in U.S. Patent No 5,489,743 and PCT application No. WO94/28122.

The present invention further includes non-human animals in which the promoter for one or more of the FGF-like polypeptides of the present invention is either activated or inactivated (*e.g.*, by using homologous recombination methods) to alter the level of expression of one or more of the native FGF-like polypeptides.

In certain embodiments, these non-human animals may be used for drug candidate screening. In such screening, the impact of a drug candidate on the animal may be measured. For example, drug candidates may decrease or increase the expression of the FGF-like gene. In certain embodiments, the amount of FGF-like polypeptide, that is produced may be measured after the exposure of the animal

to the drug candidate. Additionally, in certain embodiments, one may detect the actual impact of the drug candidate on the animal. For example, the overexpression of a particular gene may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease expression of the gene or its ability to prevent or inhibit a pathological condition. In other examples, the production of a particular metabolic product such as a fragment of a polypeptide, may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease the production of such a metabolic product or its ability to prevent or inhibit a pathological condition.

Microarray

It will be appreciated that DNA microarray technology can be utilized in accordance with the present invention. DNA microarrays are miniature, high density arrays of nucleic acids positioned on a solid support, such as glass. Each cell or element within the array has numerous copies of a single species of DNA which acts as a target for hybridization for its cognate mRNA. In expression profiling using DNA microarray technology, mRNA is first extracted from a cell or tissue sample and then converted enzymatically to fluorescently labeled cDNA. This material is hybridized to the microarray and unbound cDNA is removed by washing. The expression of discrete genes represented on the array is then visualized by quantitating the amount of labeled cDNA which is specifically bound to each target DNA. In this way, the expression of thousands of genes can be quantitated in a high throughput, parallel manner from a single sample of biological material.

This high throughput expression profiling has a broad range of applications with respect to the FGF-like polypeptides of the invention, including, but not limited to: the identification and validation of FGF-like disease-related genes as targets for therapeutics; molecular toxicology of FGF-like polypeptides and inhibitors thereof; stratification of populations and generation of surrogate markers for clinical trials; and enhancing FGF-like-related small molecule drug discovery by aiding in the identification of selective compounds in high throughput screens (HTS).

Selective Binding Agents

As used herein, the term "selective binding agent" refers to a molecule which has specificity for one or more FGF-like polypeptides. Suitable selective binding agents include, but are not limited to, antibodies and derivatives thereof, polypeptides, and small molecules. Suitable selective binding agents may be prepared using methods known in the art. An exemplary FGF-like polypeptide selective binding agent of the present invention is capable of binding a certain portion of the FGF-like polypeptide thereby inhibiting the binding of the polypeptide to the FGF-like polypeptide receptor(s).

Selective binding agents such as antibodies and antibody fragments that bind FGF-like polypeptides are within the scope of the present invention. The antibodies may be polyclonal including monospecific polyclonal, monoclonal (MAbs), recombinant, chimeric, humanized such as CDR-grafted, human, single chain, and/or bispecific, as well as fragments, variants or derivatives thereof. Antibody fragments include those portions of the antibody which bind to an epitope on the

FGF-LIKE polypeptide. Examples of such fragments include Fab and F(ab') fragments generated by enzymatic cleavage of full-length antibodies. Other binding fragments include those generated by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions.

Polyclonal antibodies directed toward an FGF-like polypeptide in certain embodiments, are produced in animals (*e.g.*, rabbits or mice) by multiple subcutaneous or intraperitoneal injections of FGF-like polypeptide and an adjuvant. It may be useful to conjugate an FGF-like polypeptide to a carrier protein that is immunogenic in the species to be immunized, such as keyhole limpet heocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum may be used to enhance the immune response. In certain embodiments, after immunization, the animals are bled and the serum is assayed for anti-FGF-like polypeptide antibody titer.

Monoclonal antibodies directed toward an FGF-like polypeptide may be produced using any method which provides for the production of antibody molecules by continuous cell lines in culture. Examples of suitable methods for preparing monoclonal antibodies include the hybridoma methods of Kohler *et al.*, *Nature*, 256:495-497 (1975) and the human B-cell hybridoma method, Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987). Also provided by the invention are hybridoma cell lines which produce monoclonal antibodies reactive with FGF-like polypeptides.

Monoclonal antibodies of the invention may be modified for use as therapeutics. One embodiment is a "chimeric" antibody in which a portion of the heavy and/or light chain is identical with or homologous to a corresponding sequence in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies, so long as they exhibit the desired biological activity. See, U.S. Patent No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci.*, 81:6851-6855 (1985).

In another embodiment, a monoclonal antibody of the invention is a "humanized" antibody. Methods for humanizing non-human antibodies are well known in the art. See U.S. Patent Nos. 5,585,089, and 5,693,762. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. Humanization can be performed, for example, using methods described in the art (Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeyen *et al.*, *Science* 239:1534-1536 (1988)), by substituting at least a portion of a rodent complementarity-determining region (CDR) for the corresponding regions of a human antibody.

Also encompassed by the invention are human antibodies which bind FGF-like polypeptides. In certain embodiments, one uses transgenic animals (*e.g.*, mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production such antibodies may be produced by immunization with an FGF-like antigen (*i.e.*, having at

least 6 contiguous amino acids), optionally conjugated to a carrier. See, for example, Jakobovits *et al.*, *Proc. Natl. Acad. Sci.*, 90:2551-2555 (1993); Jakobovits *et al.*, *Nature* 362:255-258 (1993); Bruggermann *et al.*, *Year in Immuno.*, 7:33 (1993). In certain methods, such transgenic animals are produced by incapacitating the endogenous loci encoding the heavy and light immunoglobulin chains therein, and inserting loci encoding human heavy and light chain proteins into the genome thereof. Partially modified animals, that is those having less than the full complement of modifications, are then cross-bred to obtain an animal having all of the desired immune system modifications. When administered an immunogen, these transgenic animals produce antibodies with human (rather than *e.g.*, murine) amino acid sequences, including variable regions which are immunospecific for these antigens. See PCT application nos. PCT/US96/05928 and PCT/US93/06926. Additional methods are described in U.S. Patent No. 5,545,807, PCT application nos. PCT/US91/245, PCT/GB89/01207, and in EP 546073B1 and EP 546073A1. Human antibodies may also be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

In certain embodiments, human antibodies can be produced from phage-display libraries (Hoogenboom *et al.*, *J. Mol. Biol.* 227:381 (1991); Marks *et al.*, *J. Mol. Biol.* 222:581 (1991). These processes mimic immune selection through the display of antibody repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to an antigen of choice. One such technique is described in PCT Application no. PCT/US98/17364, which describes the isolation of high affinity and functional agonistic

antibodies for MPL- and msk- receptors using such an approach.

Chimeric, CDR grafted, and humanized antibodies are typically produced by recombinant methods. Nucleic acids encoding the antibodies are introduced into host cells and expressed using materials and procedures described herein. In a certain embodiments, the antibodies are produced in mammalian host cells, such as CHO cells. Monoclonal (*e.g.*, human) antibodies may be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

The anti-FGF-like antibodies of the invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays (Sola, Monoclonal Antibodies: A Manual of Techniques, pp. 147-158 (CRC Press, Inc., 1987)) for the detection and quantitation of FGF-like polypeptides. The antibodies will bind FGF-like polypeptides with an affinity which is appropriate for the assay method being employed.

For diagnostic applications, in certain embodiments, anti-FGF-like antibodies may be labeled with a detectable moiety. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; or an enzyme, such as alkaline phosphatase, β-galactosidase, or horseradish peroxidase (Bayer *et al.*, *Meth. Enz.*, 184:138-163 (1990)).

In certain embodiments, competitive binding assays rely on the ability of a labeled standard (*e.g.*, an FGF-

like polypeptide, or an immunologically reactive portion thereof) to compete with the test sample analyte (an FGF-like polypeptide) for binding with a limited amount of anti FGF-like antibody. The amount of an FGF-like polypeptide in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, in certain embodiments, the antibodies typically are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays typically involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected and/or quantitated. In a sandwich assay, the test sample analyte is typically bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three part complex. See, *e.g.*, U.S. Patent No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assays). For example, one type of sandwich assay is an enzyme-linked immunosorbent assay (ELISA), in which case the detectable moiety is an enzyme.

The selective binding agents, including anti-FGF-like antibodies, also are useful for *in vivo* imaging. An antibody labeled with a detectable moiety may be administered to an animal, preferably into the bloodstream, and the presence and location of the labeled antibody in the host is assayed. The antibody may be labeled with any moiety that is detectable in an animal,

whether by nuclear magnetic resonance, radiology, or other detection means known in the art.

Selective binding agents of the invention, including antibodies, may be used as therapeutics. These therapeutic agents are generally agonists or antagonists, in that they either enhance or reduce, respectively, at least one of the biological activities of an FGF-like polypeptide. In one embodiment, antagonist antibodies of the invention are antibodies or binding fragments thereof which are capable of specifically binding to an FGF-like polypeptide and which are capable of inhibiting or eliminating the functional activity of an FGF-like polypeptide *in vivo* or *in vitro*. In preferred embodiments, the selective binding agent, *e.g.*, an antagonist antibody, will inhibit the functional activity of an FGF-like polypeptide by at least about 50%, and in certain embodiments, by at least about 80%. In certain embodiments, the selective binding agent may be an anti-FGF-like polypeptide antibody that is capable of interacting with an FGF-like binding partner (a ligand or receptor) thereby inhibiting or eliminating FGF-like activity *in vitro* or *in vivo*. Selective binding agents, including agonist and antagonist anti-FGF-like antibodies, are identified by screening assays which are well known in the art.

The invention also relates to a kit comprising FGF-like selective binding agents (such as antibodies) and other reagents useful for detecting FGF-like polypeptide levels in biological samples. Such reagents may include, a detectable label, blocking serum, positive and negative control samples, and detection reagents.

The FGF-like polypeptides of the present invention according to certain embodiments can be used to clone

FGF-like polypeptide receptors, using an expression cloning strategy. In certain embodiments, radiolabeled (125-Iodine) FGF-like polypeptide or affinity/activity-tagged FGF-like polypeptide (such as an Fc fusion or an alkaline phosphatase fusion) can be used in binding assays to identify a cell type or cell line or tissue that expresses FGF-like receptor(s). RNA isolated from such cells or tissues can be converted to cDNA, cloned into a mammalian expression vector, and transfected into mammalian cells (such as COS or 293 cells) to create an expression library. A radiolabeled or tagged FGF-like polypeptide can then be used as an affinity ligand to identify and isolate from this library the subset of cells which express the FGF-like receptor(s) on their surface. DNA can then be isolated from these cells and transfected into mammalian cells to create a secondary expression library in which the fraction of cells expressing FGF-like receptor(s) is many-fold higher than in the original library. This enrichment process can be repeated iteratively until a single recombinant clone containing an FGF-like receptor is isolated. Isolation of the FGF-like receptor(s) is useful for identifying or developing novel agonists and antagonists of the FGF-like polypeptide signaling pathway. Such agonists and antagonists include soluble FGF-like receptor(s), anti-FGF-like receptor antibodies, small molecules, or antisense oligonucleotides, and they may be used for treating, preventing, or diagnosing one or more disease or disorder, including those described herein.

Assaying for other modulators of FGF-like polypeptide activity

In some situations, it may be desirable to identify molecules that are modulators, *i.e.*, agonists or antagonists, of the activity of FGF-like polypeptide.

Natural or synthetic molecules that modulate FGF-like polypeptide may be identified using one or more screening assays, such as those described herein. Such molecules may be administered either in an *ex vivo* manner, or in an *in vivo* manner by injection, or by oral delivery, implantation device, or the like.

"Test molecule(s)" refers to the molecule(s) that is/are under evaluation for the ability to modulate (*i.e.*, increase or decrease) the activity of an FGF-like polypeptide. Most commonly, a test molecule will interact directly with an FGF-like polypeptide. However, it is also contemplated that a test molecule may also modulate FGF-like polypeptide activity indirectly, such as by affecting FGF-like gene expression, or by binding to an FGF-like binding partner (*e.g.*, receptor or ligand). In certain embodiments, a test molecule will bind to an FGF-like polypeptide with an affinity constant of at least about 10^{-6} M, preferably about 10^{-8} M, more preferably about 10^{-9} M, and even more preferably about 10^{-10} M.

Methods for identifying compounds which interact with FGF-like polypeptides are encompassed by the present invention. In certain embodiments, an FGF-like polypeptide is incubated with a test molecule under conditions which permit the interaction of the test molecule with an FGF-like polypeptide, and the extent of the interaction can be measured. The test molecule(s) can be screened in a substantially purified form or in a crude mixture.

In certain embodiments, an FGF-like polypeptide agonist or antagonist may be a protein, peptide, carbohydrate, lipid, or small molecular weight molecule which interacts with FGF-like polypeptide to regulate its

activity. Molecules which regulate FGF-like polypeptide expression include nucleic acids which are complementary to nucleic acids encoding an FGF-like polypeptide, or are complementary to nucleic acids sequences which direct or control the expression of FGF-like polypeptide, and which act as anti-sense regulators of expression.

Once a set of test molecules has been identified as interacting with an FGF-like polypeptide, the molecules may be further evaluated for their ability to increase or decrease FGF-like polypeptide activity. The measurement of the interaction of test molecules with FGF-like polypeptides may be carried out in several formats, including cell-based binding assays, membrane binding assays, solution-phase assays and immunoassays. Typically, test molecules are incubated with an FGF-like polypeptide for a specified period of time, and FGF-like polypeptide activity is determined by one or more assays for measuring biological activity.

The interaction of test molecules with FGF-like polypeptides may also be assayed directly using polyclonal or monoclonal antibodies in an immunoassay. Alternatively, modified forms of FGF-like polypeptides containing epitope tags as described herein may be used in immunoassays.

In the event that FGF-like polypeptides display biological activity through an interaction with a binding partner (*e.g.*, a receptor or a ligand), a variety of *in vitro* assays may be used to measure the binding of an FGF-like polypeptide to the corresponding binding partner (such as a selective binding agent, receptor, or ligand). These assays may be used to screen test molecules for their ability to increase or decrease the rate and/or the extent of binding of an FGF-like polypeptide to its

binding partner. In one assay, an FGF-like polypeptide is immobilized in the wells of a microtiter plate. Radiolabeled FGF-like binding partner (for example, iodinated FGF-like binding partner) and the test molecule(s) can then be added either one at a time (in either order) or simultaneously to the wells. After incubation, the wells can be washed and counted, using a scintillation counter, for radioactivity to determine the extent to which the binding partner bound to FGF-like polypeptide. Typically, the molecules will be tested over a range of concentrations, and a series of control wells lacking one or more elements of the test assays can be used for accuracy in the evaluation of the results. An alternative to this method involves reversing the "positions" of the proteins, *i.e.*, immobilizing FGF-like binding partner to the microtiter plate wells, incubating with the test molecule and radiolabeled FGF-like polypeptide, and determining the extent of FGF-like polypeptide binding. See, for example, chapter 18, *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, New York, NY (1995).

As an alternative to radiolabelling, an FGF-like polypeptide or its binding partner may be conjugated to biotin and the presence of biotinylated protein can then be detected using streptavidin linked to an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase (AP), that can be detected colorometrically, or by fluorescent tagging of streptavidin. An antibody directed to an FGF-like polypeptide or to an FGF-like binding partner and conjugated to biotin may also be used and can be detected after incubation with enzyme-linked streptavidin linked to AP or HRP.

An FGF-like polypeptide or an FGF-like binding partner can also be immobilized by attachment to agarose

beads, acrylic beads or other types of such inert solid phase substrates. The substrate-protein complex can be placed in a solution containing the complementary protein and the test compound. After incubation, the beads can be precipitated by centrifugation, and the amount of binding between an FGF-like polypeptide and its binding partner can be assessed using the methods described herein. Alternatively, the substrate-protein complex can be immobilized in a column, and the test molecule and complementary protein are passed through the column. The formation of a complex between an FGF-like polypeptide and its binding partner can then be assessed using any of the techniques set forth herein, *i.e.*, radiolabelling, antibody binding, or the like.

Another *in vitro* assay that is useful for identifying a test molecule which increases or decreases the formation of a complex between an FGF-like binding protein and an FGF-like binding partner is a surface plasmon resonance detector system such as the BIAcore assay system (Pharmacia, Piscataway, NJ). The BIAcore system may be carried out using the manufacturer's protocol. This assay essentially involves the covalent binding of either FGF-like polypeptide or an FGF-like binding partner to a dextran-coated sensor chip which is located in a detector. The test compound and the other complementary protein can then be injected, either simultaneously or sequentially, into the chamber containing the sensor chip. The amount of complementary protein that binds can be assessed based on the change in molecular mass which is physically associated with the dextran-coated side of the sensor chip; the change in molecular mass can be measured by the detector system.

In some cases, it may be desirable to evaluate two or more test compounds together for their ability to

increase or decrease the formation of a complex between an FGF-like polypeptide and an FGF-like binding partner. In these cases, the assays set forth herein can be readily modified by adding such additional test compound(s) either simultaneous with, or subsequent to, the first test compound. The remainder of the steps in the assay are as set forth herein.

In vitro assays such as those described herein may be used advantageously to screen large numbers of compounds for effects on complex formation by FGF-like polypeptide and FGF-like binding partner. The assays may be automated to screen compounds generated in phage display, synthetic peptide, and chemical synthesis libraries.

Compounds which increase or decrease the formation of a complex between an FGF-like polypeptide and an FGF-like binding partner may also be screened in cell culture using cells and cell lines expressing either FGF-like polypeptide or FGF-like binding partner. Cells and cell lines may be obtained from any mammal, but preferably will be from human or other primate, canine, or rodent sources. The binding of an FGF-like polypeptide to cells expressing FGF-like binding partner at the surface is evaluated in the presence or absence of test molecules, and the extent of binding may be determined by, for example, flow cytometry using a biotinylated antibody to an FGF-like binding partner. Cell culture assays can be used advantageously to further evaluate compounds that score positive in protein binding assays described herein.

Cell cultures can also be used to screen the impact of a drug candidate. For example, drug candidates may decrease or increase the expression of the FGF-like gene.

In certain embodiments, the amount of FGF-like polypeptide that is produced may be measured after exposure of the cell culture to the drug candidate. In certain embodiments, one may detect the actual impact of the drug candidate on the cell culture. For example, the overexpression of a particular gene may have a particular impact on the cell culture. In such cases, one may test a drug candidate's ability to increase or decrease the expression of the gene or its ability to prevent or inhibit a particular impact on the cell culture. In other examples, the production of a particular metabolic product such as a fragment of a polypeptide, may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease the production of such a metabolic product in a cell culture.

Internalizing Proteins

In certain embodiments, the tat protein sequence (from HIV) can be used to internalize proteins into a cell. See e.g., Falwell et al., Proc. Natl. Acad. Sci., 91:664-668 (1994). For example, an 11 amino acid sequence (YGRKKRRQRRR) of the HIV tat protein (termed the "protein transduction domain", or TAT PDT) has been described as mediating delivery across the cytoplasmic membrane and the nuclear membrane of a cell. See Schwarze et al., Science, 285:1569-1572 (1999); and Nagahara et al., Nature Medicine, 4:1449-1452 (1998). In these procedures, FITC-constructs (FITC-GGGGYGRKKRRQRRR) are prepared which bind to cells as observed by fluorescence-activated cell sorting (FACS) analysis, and these constructs penetrate tissues after i.p. adminstration. Next, tat-bgal fusion proteins are constructed. Cells treated with this construct demonstrated b-gal activity. Following injection, a

number of tissues, including liver, kidney, lung, heart, and brain tissue have been found to demonstrate expression using these procedures. It is believed that these constructions underwent some degree of unfolding in order to enter the cell; as such, refolding may be required after entering the cell.

It will thus be appreciated that the tat protein sequence may be used to internalize a desired protein or polypeptide into a cell. For example, using the tat protein sequence, an FGF-like antagonist (such as an anti-FGF-like selective binding agent, small molecule, soluble receptor, or antisense oligonucleotide) can be administered intracellularly to inhibit the activity of an FGF-like molecule. As used herein, the term "FGF-like molecule" refers to both FGF-like nucleic acid molecules and FGF-like polypeptides as defined herein. Where desired, the FGF-like protein itself may also be internally administered to a cell using these procedures. See also, Strauss, E., "Introducing Proteins Into the Body's Cells", Science, 285:1466-1467 (1999).

Therapeutic Uses

The FGF-like polypeptides of this invention exhibit similar activities and may be useful for the same purposes as known members of the FGF family of polypeptides. Thus, the FGF-like polypeptides of this invention may be potent mitogens for a variety of cells of the mesodermal, exodermal and endodermal origin, including fibroblasts, corneal and vascular endothelial cells, granulocytes, adrenal cortical cells, chondrocytes, myoblasts, vascular smooth muscle cells, lens epithelial cells, retinal cells, melanocytes, keratinocytes, oligodendrocytes, astrocytes, osteoblasts, renal cells and hematopoietic cells. Included among

these biological activities are the ability to stimulate the proliferation and/or differentiation of liver cells (e.g., hepatocytes), and these polypeptides may therefore have utility in differentiating liver cells from background. Another activity attributable to the polypeptides of this invention may be the ability to stimulate the proliferation of vascular endothelial cells and to enable endothelial cells to penetrate the basement membrane. Consistent with these properties, the FGF-like polypeptides of this invention may possess the ability to stimulate angiogenesis and to promote wound healing (i.e., facilitate the repair or replacement of damages of diseased tissue resulting from burns, traumatic injuries, surgery, ulcers, etc.). These polypeptides may also induce mesoderm formation and modulate the differentiation of neuronal cells, adipocytes and skeleton muscle cells. The polypeptides may also be employed to prevent or ameliorate skin aging due to sun exposure by stimulating keratinocyte growth. Further, the polypeptides of this invention may be employed to maintain organs before transplantation or for supporting cultures of primary cells and tissues. In addition, these polypeptides may be utilized to prevent hair loss since FGF family members activate hair-forming cells and promote melanocyte growth. They may also be used to stimulate the growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

The polypeptides of this invention may also be useful as fat deposition inhibitors, and, therefore, they may be applicable for the treatment of obesity or diabetes.

A non-exclusive list of acute and chronic conditions, disorders or diseases which can be treated,

diagnosed, or prevented with the polypeptides and nucleic acids of the invention include: dermal wounds, epidermolysis bullosa, male pattern alopecia, gastric ulcer, duodenal ulcer, erosive gastritis, esophagitis, esophageal reflux disease, inflammatory bowel disease or Crohn's disease, radiation- or chemotherapy-induced gut toxicity, hyaline membrane disease, necrosis of the respiratory epithelium, emphysema, pulmonary inflammation, pulmonary fibrosis, hepatic cirrhosis or toxic insults to the liver, fulminant liver failure, viral hepatitis, mucositis, multiple sclerosis and other neurodegenerative diseases, infantile respiratory distress syndrome, bronchopulmonary displasia, acute respiratory distress syndrome or other lung abnormalities, or tumors of the eye or other tissues and organs.

FGF-like Compositions and Administration

Therapeutic compositions are within the scope of the present invention. Such FGF-like pharmaceutical compositions may comprise a therapeutically effective amount of an FGF-like polypeptide or an FGF-like nucleic acid molecule in admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration. Pharmaceutical compositions may comprise a therapeutically effective amount of one or more FGF-like selective binding agents in admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration.

Acceptable formulation materials preferably are nontoxic to recipients at the dosages and concentrations employed.

The pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine), antimicrobials, antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite), buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin), fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrins), proteins (such as serum albumin, gelatin or immunoglobulins), coloring, flavoring and diluting agents, emulsifying agents, hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide), solvents (such as glycerin, propylene glycol or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol), suspending agents, surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal), stability enhancing agents (sucrose or sorbitol), tonicity enhancing agents (such as alkali metal halides (preferably sodium or potassium chloride), mannitol

sorbitol), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants. (*Remington's Pharmaceutical Sciences*, 18th Edition, A.R. Gennaro, ed., Mack Publishing Company [1990]).

The optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage. See for example, *Remington's Pharmaceutical Sciences, supra*. Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the FGF-like molecule.

The primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor. In one embodiment of the present invention, FGF-like polypeptide compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (*Remington's Pharmaceutical Sciences, supra*) in the form of a lyophilized cake or an aqueous solution. Further, the FGF-like polypeptide product may be formulated as a lyophilizate using appropriate excipients such as sucrose.

The FGF-like pharmaceutical compositions can be

selected for parenteral delivery. Alternatively, the compositions may be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art.

The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at slightly lower pH, typically within a pH range of from about 5 to about 8.

When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the desired FGF-like molecule in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which a FGF-like molecule is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), or beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered as a depot injection. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

In one embodiment, a pharmaceutical composition may be formulated for inhalation. For example, an FGF-like molecule may be formulated as a dry powder for inhalation. FGF-like polypeptide or FGF-like nucleic acid molecule inhalation solutions may also be formulated

with a propellant for aerosol delivery. In yet another embodiment, solutions may be nebulized. Pulmonary administration is further described in PCT application no. PCT/US94/001875, which describes pulmonary delivery of chemically modified proteins.

It is also contemplated that certain formulations may be administered orally. In one embodiment of the present invention, FGF-like molecules that are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the FGF-like molecule. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

Another pharmaceutical composition may involve an effective quantity of FGF-like molecules in a mixture with non-toxic excipients that are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or other appropriate vehicle, solutions can be prepared in unit dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

Additional FGF-like pharmaceutical compositions will be evident to those skilled in the art, including formulations involving FGF-like polypeptides in

sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See for example, PCT/US93/00829 that describes controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions. Additional examples of sustained-release preparations include semi-permeable polymer matrices in the form of shaped articles, *e.g.* films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, *Biopolymers*, 22:547-556 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, *J. Biomed. Mater. Res.*, 15:167-277 (1981) and Langer, *Chem. Tech.*, 12:98-105 (1982)), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also may include liposomes, which can be prepared by any of several methods known in the art. See *e.g.*, Eppstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688-3692 (1985); EP 36,676; EP 88,046; EP 143,949.

The FGF-like pharmaceutical composition to be used for *in vivo* administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using these methods may be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port,

for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (*e.g.*, lyophilized) requiring reconstitution prior to administration.

In a specific embodiment, the present invention is directed to kits for producing a single-dose administration unit. The kits may each contain both a first container having a dried protein and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (*e.g.*, liquid syringes and lyosyringes).

An effective amount of an FGF-like pharmaceutical composition to be employed therapeutically may depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the FGF-like molecule is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 µg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage may range from 0.1 µg/kg up to about 100 mg/kg; or 1 µg/kg up to about 100 mg/kg; or 5 µg/kg up to about 100 mg/kg.

The frequency of dosing may depend upon the pharmacokinetic parameters of the FGF-like molecule in the formulation used. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages may be ascertained through use of appropriate dose-response data.

The route of administration of the pharmaceutical composition is in accord with known methods, *e.g.* oral, injection by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intra-ocular, intraarterial, intraportal, or intralesional routes, or by sustained release systems or implantation device. Where desired, the compositions may be administered by bolus injection or continuously by infusion, or by implantation device.

Alternatively or additionally, the composition may be administered locally via implantation of a membrane, sponge, or other appropriate material on to which the desired molecule has been absorbed or encapsulated. Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of the desired molecule may be via diffusion, timed release bolus, or continuous administration.

In some cases, it may be desirable to use FGF-like pharmaceutical compositions in an *ex vivo* manner. In

such instances, cells, tissues, or organs that have been removed from the patient are exposed to FGF-like pharmaceutical compositions after which the cells, tissues and/or organs are subsequently implanted back into the patient.

In other cases, an FGF-like polypeptide can be delivered by implanting certain cells that have been genetically engineered, using methods such as those described herein, to express and secrete the polypeptide. Such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. Optionally, the cells may be immortalized. In order to decrease the chance of an immunological response, the cells may be encapsulated to avoid infiltration of surrounding tissues. The encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

Additional embodiments of the present invention relate to cells and methods (*e.g.*, homologous recombination and/or other recombinant production methods) for both the *in vitro* production of therapeutic polypeptides and for the production and delivery of therapeutic polypeptides by gene therapy or cell therapy. Homologous and other recombination methods may be used to modify a cell that contains a normally transcriptionally silent FGF-like gene, or an under expressed gene, and thereby produce a cell which expresses therapeutically efficacious amounts of FGF-like polypeptides.

Homologous recombination is a technique originally developed for targeting genes to induce or correct mutations in transcriptionally active genes

(Kucherlapati, *Prog. in Nucl. Acid Res. & Mol. Biol.*, 36:301, 1989). The basic technique was developed as a method for introducing specific mutations into specific regions of the mammalian genome (Thomas *et al.*, *Cell*, 44:419-428, 1986; Thomas and Capecchi, *Cell*, 51:503-512, 1987; Doetschman *et al.*, *Proc. Natl. Acad. Sci.*, 85:8583-8587, 1988) or to correct specific mutations within defective genes (Doetschman *et al.*, *Nature*, 330:576-578, 1987). Exemplary homologous recombination techniques are described in U.S. Patent No. 5,272,071 (EP 9193051, EP Publication No. 505500; PCT/US90/07642, International Publication No. WO 91/09955).

Through homologous recombination, the DNA sequence to be inserted into the genome can be directed to a specific region of the gene of interest by attaching it to targeting DNA. The targeting DNA is a nucleotide sequence that is complementary (homologous) to a region of the genomic DNA. Small pieces of targeting DNA that are complementary to a specific region of the genome are put in contact with the parental strand during the DNA replication process. It is a general property of DNA that has been inserted into a cell to hybridize, and therefore, recombine with other pieces of endogenous DNA through shared homologous regions. If this complementary strand is attached to an oligonucleotide that contains a mutation or a different sequence or an additional nucleotide, it too is incorporated into the newly synthesized strand as a result of the recombination. As a result of the proofreading function, it is possible for the new sequence of DNA to serve as the template. Thus, the transferred DNA is incorporated into the genome.

Attached to these pieces of targeting DNA are regions of DNA which may interact with or control the expression of a FGF-like polypeptide, *e.g.*, flanking

sequences. For example, a promoter/enhancer element, a suppresser, or an exogenous transcription modulatory element is inserted in the genome of the intended host cell in proximity and orientation sufficient to influence the transcription of DNA encoding the desired FGF-like polypeptide. The control element controls a portion of the DNA present in the host cell genome. Thus, the expression of the desired FGF-like polypeptide may be achieved not by transfection of DNA that encodes the FGF-like gene itself, but rather by the use of targeting DNA (containing regions of homology with the endogenous gene of interest) coupled with DNA regulatory segments that provide the endogenous gene sequence with recognizable signals for transcription of an FGF-like polypeptide.

In an exemplary method, the expression of a desired targeted gene in a cell (*i.e.*, a desired endogenous cellular gene) is altered via homologous recombination into the cellular genome at a preselected site, by the introduction of DNA that includes at least a regulatory sequence, an exon and a splice donor site. These components are introduced into the chromosomal (genomic) DNA in such a manner that this, in effect, results in the production of a new transcription unit (in which the regulatory sequence, the exon and the splice donor site present in the DNA construct are operatively linked to the endogenous gene). As a result of the introduction of these components into the chromosomal DNA, the expression of the desired endogenous gene is altered.

Altered gene expression, as described herein, encompasses activating (or causing to be expressed) a gene which is normally silent (unexpressed) in the cell as obtained, as well as increasing the expression of a gene which is not expressed at physiologically significant levels in the cell as obtained. The

embodiments further encompass changing the pattern of regulation or induction such that it is different from the pattern of regulation or induction that occurs in the cell as obtained, and reducing (including eliminating) the expression of a gene which is expressed in the cell as obtained.

One method by which homologous recombination can be used to increase, or cause, FGF-like polypeptide production from a cell's endogenous FGF-like gene involves first using homologous recombination to place a recombination sequence from a site-specific recombination system (*e.g.*, Cre/loxP, FLP/FRT) (Sauer, *Current Opinion In Biotechnology*, 5:521-527, 1994; Sauer, *Methods In Enzymology*, 225:890-900, 1993) upstream (that is, 5' to) of the cell's endogenous genomic FGF-like polypeptide coding region. A plasmid containing a recombination site homologous to the site that was placed just upstream of the genomic FGF-like polypeptide coding region is introduced into the modified cell line along with the appropriate recombinase enzyme. This recombinase causes the plasmid to integrate, via the plasmid's recombination site, into the recombination site located just upstream of the genomic FGF-like polypeptide coding region in the cell line (Baubonis and Sauer, *Nucleic Acids Res.*, 21:2025-2029, 1993; O'Gorman *et al.*, *Science*, 251:1351-1355, 1991). Any flanking sequences known to increase transcription (*e.g.*, enhancer/promoter, intron, translational enhancer), if properly positioned in this plasmid, would integrate in such a manner as to create a new or modified transcriptional unit resulting in *de novo* or increased FGF-like polypeptide production from the cell's endogenous FGF-like gene.

A further method to use the cell line in which the site specific recombination sequence had been placed just

upstream of the cell's endogenous genomic FGF-like polypeptide coding region is to use homologous recombination to introduce a second recombination site elsewhere in the cell line's genome. The appropriate recombinase enzyme is then introduced into the two-recombination-site cell line, causing a recombination event (deletion, inversion, translocation) (Sauer, *Current Opinion In Biotechnology, supra*, 1994; Sauer, *Methods In Enzymology, supra*, 1993) that would create a new or modified transcriptional unit resulting in *de novo* or increased FGF-like polypeptide production from the cell's endogenous FGF-like gene.

An additional approach for increasing, or causing, the expression of FGF-like polypeptide from a cell's endogenous FGF-like gene involves increasing, or causing, the expression of a gene or genes (*e.g.*, transcription factors) and/or decreasing the expression of a gene or genes (*e.g.*, transcriptional repressors) in a manner which results in *de novo* or increased FGF-like polypeptide production from the cell's endogenous FGF-like gene. This method includes the introduction of a non-naturally occurring polypeptide (*e.g.*, a polypeptide comprising a site specific DNA binding domain fused to a transcriptional factor domain) into the cell such that *de novo* or increased FGF-like polypeptide production from the cell's endogenous FGF-like gene results.

The present invention further relates to DNA constructs useful in the method of altering expression of a target gene. In certain embodiments, the exemplary DNA constructs comprise: (a) one or more targeting sequences; (b) a regulatory sequence; (c) an exon; and (d) an unpaired splice-donor site. The targeting sequence in the DNA construct directs the integration of elements (a)-(d) into a target gene in a cell such that

the elements (b)-(d) are operatively linked to sequences of the endogenous target gene. In another embodiment, the DNA constructs comprise: (a) one or more targeting sequences, (b) a regulatory sequence, (c) an exon, (d) a splice-donor site, (e) an intron, and (f) a splice-acceptor site, wherein the targeting sequence directs the integration of elements (a)-(f) such that the elements of (b)-(f) are operatively linked to the endogenous gene. The targeting sequence is homologous to the preselected site in the cellular chromosomal DNA with which homologous recombination is to occur. In the construct, the exon is generally 3' of the regulatory sequence and the splice-donor site is 3' of the exon.

If the sequence of a particular gene is known, such as the nucleic acid sequence of FGF-like polypeptide presented herein, a piece of DNA that is complementary to a selected region of the gene can be synthesized or otherwise obtained, such as by appropriate restriction of the native DNA at specific recognition sites bounding the region of interest. This piece serves as a targeting sequence(s) upon insertion into the cell and will hybridize to its homologous region within the genome. If this hybridization occurs during DNA replication, this piece of DNA, and any additional sequence attached thereto, will act as an Okazaki fragment and will be incorporated into the newly synthesized daughter strand of DNA. The present invention, therefore, includes nucleotides encoding a FGF-like polypeptide, which nucleotides may be used as targeting sequences.

FGF-like polypeptide cell therapy, *e.g.*, the implantation of cells producing FGF-like polypeptides, is also contemplated. This embodiment involves implanting cells capable of synthesizing and secreting a biologically active form of FGF-like polypeptide. Such

FGF-like polypeptide-producing cells can be cells that are natural producers of FGF-like polypeptides or may be recombinant cells whose ability to produce FGF-like polypeptides has been augmented by transformation with a gene encoding the desired FGF-like polypeptide or with a gene augmenting the expression of FGF-like polypeptide. Such a modification may be accomplished by means of a vector suitable for delivering the gene as well as promoting its expression and secretion. In order to minimize a potential immunological reaction in patients being administered an FGF-like polypeptide, as may occur with the administration of a polypeptide of a foreign species, it is preferred that the natural cells producing FGF-like polypeptide be of human origin and produce human FGF-like polypeptide. Likewise, it is preferred that the recombinant cells producing FGF-like polypeptide be transformed with an expression vector containing a gene encoding a human FGF-like polypeptide.

Implanted cells may be encapsulated to avoid the infiltration of surrounding tissue. Human or non-human animal cells may be implanted in patients in biocompatible, semipermeable polymeric enclosures or membranes that allow the release of FGF-like polypeptide, but that prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissue. Alternatively, the patient's own cells, transformed to produce FGF-like polypeptides *ex vivo*, may be implanted directly into the patient without such encapsulation.

Techniques for the encapsulation of living cells are known in the art, and the preparation of the encapsulated cells and their implantation in patients may be routinely accomplished. For example, Baetge *et al.* (WO95/05452; PCT/US94/09299) describe membrane capsules containing

genetically engineered cells for the effective delivery of biologically active molecules. The capsules are biocompatible and are easily retrievable. The capsules encapsulate cells transfected with recombinant DNA molecules comprising DNA sequences coding for biologically active molecules operatively linked to promoters that are not subject to down regulation *in vivo* upon implantation into a mammalian host. The devices provide for the delivery of the molecules from living cells to specific sites within a recipient. In addition, see U.S. Patent Nos. 4,892,538, 5,011,472, and 5,106,627. A system for encapsulating living cells is described in PCT Application no. PCT/US91/00157 of Aebischer *et al.* See also, PCT Application no. PCT/US91/00155 of Aebischer *et al.*, Winn *et al.*, *Exper. Neurol.*, 113:322-329 (1991), Aebischer *et al.*, *Exper. Neurol.*, 111:269-275 (1991); and Tresco *et al.*, ASAIO, 38:17-23 (1992).

In vivo and *in vitro* gene therapy delivery of FGF-like polypeptides is also envisioned. One example of a gene therapy technique is to use the FGF-like gene (either genomic DNA, cDNA, and/or synthetic DNA) encoding a FGF-like polypeptide which may be operably linked to a constitutive or inducible promoter to form a "gene therapy DNA construct". The promoter may be homologous or heterologous to the endogenous FGF-like gene, provided that it is active in the cell or tissue type into which the construct will be inserted. Other components of the gene therapy DNA construct may optionally include, DNA molecules designed for site-specific integration (*e.g.*, endogenous sequences useful for homologous recombination), tissue-specific promoter, enhancer(s) or silencer(s), DNA molecules capable of providing a selective advantage over the parent cell, DNA molecules useful as labels to identify transformed cells, negative selection systems, cell specific binding agents (as, for

example, for cell targeting), cell-specific internalization factors, and transcription factors to enhance expression by a vector as well as factors to enable vector manufacture.

A gene therapy DNA construct can then be introduced into cells (either *ex vivo* or *in vivo*) using viral or non-viral vectors. One means for introducing the gene therapy DNA construct is by means of viral vectors as described herein. Certain vectors, such as retroviral vectors, will deliver the DNA construct to the chromosomal DNA of the cells, and the gene can integrate into the chromosomal DNA. Other vectors will function as episomes, and the gene therapy DNA construct will remain in the cytoplasm.

In yet other embodiments, regulatory elements can be included for the controlled expression of the FGF-like gene in the target cell. Such elements are turned on in response to an appropriate effector. In this way, a therapeutic polypeptide can be expressed when desired. One conventional control means involves the use of small molecule dimerizers or rapalogs (as described in WO9641865 (PCT/US96/099486); WO9731898 (PCT/US97/03137) and WO9731899 (PCT/US95/03157) used to dimerize chimeric proteins which contain a small molecule-binding domain and a domain capable of initiating biological process, such as a DNA-binding protein or transcriptional activation protein. The dimerization of the proteins can be used to initiate transcription of the transgene.

An alternative regulation technology uses a method of storing proteins expressed from the gene of interest inside the cell as an aggregate or cluster. The gene of interest is expressed as a fusion protein that includes a conditional aggregation domain which results in the

retention of the aggregated protein in the endoplasmic reticulum. The stored proteins are stable and inactive inside the cell. The proteins can be released, however, by administering a drug (*e.g.*, small molecule ligand) that removes the conditional aggregation domain and thereby specifically breaks apart the aggregates or clusters so that the proteins may be secreted from the cell. See, *Science* 287:816-817, and 826-830 (2000).

Other suitable control means or gene switches include, but are not limited to, the following systems. Mifepristone (RU486) is used as a progesterone antagonist. The binding of a modified progesterone receptor ligand-binding domain to the progesterone antagonist activates transcription by forming a dimer of two transcription factors which then pass into the nucleus to bind DNA. The ligand binding domain is modified to eliminate the ability of the receptor to bind to the natural ligand. The modified steroid hormone receptor system is further described in U.S. 5,364,791; WO9640911, and WO9710337.

Yet another control system uses ecdysone (a fruit fly steroid hormone) which binds to and activates an ecdysone receptor (cytoplasmic receptor). The receptor then translocates to the nucleus to bind a specific DNA response element (promoter from ecdysone-responsive gene). The ecdysone receptor includes a transactivation domain/DNA-binding domain/ligand-binding domain to initiate transcription. The ecdysone system is further described in U.S. 5,514,578; WO9738117; WO9637609; and WO9303162.

Another control system uses a positive tetracycline-controllable transactivator. This system involves a mutated tet repressor protein DNA-binding domain (mutated

tet R-4 amino acid changes which resulted in a reverse tetracycline-regulated transactivator protein, *i.e.*, it binds to a tet operator in the presence of tetracycline) linked to a polypeptide which activates transcription. Such systems are described in U.S. Patent Nos. 5,464,758; 5,650,298 and 5,654,168.

Additional expression control systems and nucleic acid constructs are described in U.S. Patent Nos. 5,741,679 and 5,834,186, to Innovir Laboratories Inc.

In vivo gene therapy may be accomplished by introducing the gene encoding an FGF-like polypeptide into cells via local injection of an FGF-like nucleic acid molecule or by other appropriate viral or non-viral delivery vectors. Hefti, *Neurobiology*, 25:1418-1435 (1994). For example, a nucleic acid molecule encoding an FGF-like polypeptide may be contained in an adeno-associated virus (AAV) vector for delivery to the targeted cells (*e.g.*, Johnson, International Publication No. WO95/34670; International Application No. PCT/US95/07178). The recombinant AAV genome typically contains AAV inverted terminal repeats flanking a DNA sequence encoding an FGF-like polypeptide operably linked to functional promoter and polyadenylation sequences.

Alternative suitable viral vectors include, but are not limited to, retrovirus, adenovirus, herpes simplex virus, lentivirus, hepatitis virus, parvovirus, papovavirus, poxvirus, alphavirus, coronavirus, rhabdovirus, paramyxovirus, and papilloma virus vectors. U.S. Patent No. 5,672,344 describes an *in vivo* viral-mediated gene transfer system involving a recombinant neurotrophic HSV-1 vector. U.S. Patent No. 5,399,346 provides examples of a process for providing a patient with a therapeutic protein by the delivery of human cells

that have been treated *in vitro* to insert a DNA segment encoding a therapeutic protein. Additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent No. 5,631,236 involving adenoviral vectors; U.S. Patent No. 5,672,510 involving retroviral vectors; and U.S. 5,635,399 involving retroviral vectors expressing cytokines.

Nonviral delivery methods include, but are not limited to, liposome-mediated transfer, naked DNA delivery (direct injection), receptor-mediated transfer (ligand-DNA complex), electroporation, calcium phosphate precipitation, and microparticle bombardment (*e.g.*, gene gun). Gene therapy materials and methods may also include the use of inducible promoters, tissue-specific enhancer-promoters, DNA sequences designed for site-specific integration, DNA sequences capable of providing a selective advantage over the parent cell, labels to identify transformed cells, negative selection systems and expression control systems (safety measures), cell-specific binding agents (for cell targeting), cell-specific internalization factors, and transcription factors to enhance expression by a vector as well as methods of vector manufacture. Such additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent No. 4,970,154 involving electroporation techniques; WO96/40958 involving nuclear ligands; U.S. Patent No. 5,679,559 describing a lipoprotein-containing system for gene delivery; U.S. Patent No. 5,676,954 involving liposome carriers; U.S. Patent No. 5,593,875 concerning methods for calcium phosphate transfection; and U.S. Patent No. 4,945,050 wherein biologically active particles are propelled at cells at a speed whereby the particles penetrate the surface of the cells and become incorporated into the interior of the cells.

It is also contemplated that FGF-like gene therapy or cell therapy can further include the delivery of one or more additional polypeptide(s) in the same or a different cell(s). Such cells may be separately introduced into the patient, or the cells may be contained in a single implantable device, such as the encapsulating membrane described above, or the cells may be separately modified by means of viral vectors.

A way to increase endogenous FGF-like polypeptide expression in a cell via gene therapy is to insert one or more enhancer elements into the FGF-like polypeptide promoter, where the enhancer element(s) can serve to increase transcriptional activity of the FGF-like gene. The enhancer element(s) used will be selected based on the tissue in which one desires to activate the gene(s); enhancer elements known to confer promoter activation in that tissue will be selected. For example, if a gene encoding a FGF-like polypeptide is to be "turned on" in T-cells, the *lck* promoter enhancer element may be used. Here, the functional portion of the transcriptional element to be added may be inserted into a fragment of DNA containing the FGF-like polypeptide promoter (and optionally, inserted into a vector and/or 5' and/or 3' flanking sequence(s), etc.) using standard cloning techniques. This construct, known as a "homologous recombination construct", can then be introduced into the desired cells either *ex vivo* or *in vivo*.

Gene therapy also can be used to decrease FGF-like polypeptide expression by modifying the nucleotide sequence of the endogenous promoter(s). Such modification is typically accomplished via homologous recombination methods. For example, a DNA molecule containing all or a portion of the promoter of the FGF-like gene(s) selected for inactivation can be engineered to remove and/or replace pieces of the promoter that

regulate transcription. For example the TATA box and/or the binding site of a transcriptional activator of the promoter may be deleted using standard molecular biology techniques; such deletion can inhibit promoter activity thereby repressing the transcription of the corresponding FGF-like gene. The deletion of the TATA box or the transcription activator binding site in the promoter may be accomplished by generating a DNA construct comprising all or the relevant portion of the FGF-like polypeptide promoter(s) (from the same or a related species as the FGF-like gene(s) to be regulated) in which one or more of the TATA box and/or transcriptional activator binding site nucleotides are mutated via substitution, deletion and/or insertion of one or more nucleotides. As a result, the TATA box and/or activator binding site has decreased activity or is rendered completely inactive. The construct will typically contain at least about 500 bases of DNA that correspond to the native (endogenous) 5' and 3' DNA sequences adjacent to the promoter segment that has been modified. The construct may be introduced into the appropriate cells (either *ex vivo* or *in vivo*) either directly or via a viral vector as described herein. Typically, the integration of the construct into the genomic DNA of the cells will be via homologous recombination, where the 5' and 3' DNA sequences in the promoter construct can serve to help integrate the modified promoter region via hybridization to the endogenous chromosomal DNA.

Phenotypes that were observed after overexpression of FGF-like polypeptide in transgenic mice suggest at least one activity of FGF-like polypeptide related to the development, stimulation, and/or repair of multiple epithelial tissues. Thus, FGF-like polypeptide may be used in multiple therapeutic treatments.

Thus, according to certain embodiments, FGF-like polypeptide may be used for conditions involving tissues characterized by damage to or deficiencies in epithelial cells. According to certain embodiments, FGF-like polypeptide may be used for diseases or medical conditions as discussed below. In view of the transgenic mouse data that shows an impact on epithelial cells and in view of the structural similarity of human FGF-like polypeptide to human KGF molecules, human FGF-like polypeptides may be used for the same or similar indications as KGF molecules may be used.

Stimulation of proliferation and differentiation of adnexal structures such as hair follicles, sweat glands, and sebaceous glands is typically important in regenerating epidermis and dermis in patients with burns and other partial and full thickness injuries. At present, surface defects can heal by scar formation and keratinocyte resurfacing. The use of FGF-like polypeptide, according to certain embodiments, may result in repopulating hair follicles, sweat glands, and sebaceous glands in partial or full thickness skin defects, including burns. Standard in vivo models of adnexal structure proliferation and stimulation which permit the predictive testing of compounds having human therapeutic efficacy for burns and other partial and full-thickness injuries are well-known. Mustoe, et al. (1991), "Growth Factor-Induced Acceleration of Tissue Repair through Direct and Inductive Activities in a Rabbit Dermal Ulcer Model", *J. Clin. Invest.*, 87:694-703; Pierce, et al., "Platelet-derived Growth Factor (BB Homodimer), Transforming Growth Factor β-1, and Basic Fibroblast Growth Factor in Dermal Wound Healing", *American Journal of Pathology*, 140(6):1375 (1992)); and

Davis, et al., "Second-Degree Burn Healing: The Effect of Occlusive Dressings and a Cream", *Journal of Surgical Research*, 48:245-248 (1990).

Epidermolysis bullosa is a defect in adherence of the epidermis to the underlying dermis, resulting in frequent open, painful blisters which can cause severe morbidity. Accelerated reepithelialization of these lesions may result in less risk of infection, diminished pain, and less wound care. According to certain embodiments, FGF-like polypeptide may be useful for such treatment.

Chemotherapy-induced alopecia results when patients are treated with courses of chemotherapy for malignancy. It would be useful to have a therapeutic effective at preventing the hair follicle cells from death, which cause the transient loss of hair. According to certain embodiments, FGF-like polypeptide may be useful for such treatment. Standard in vivo models of chemotherapy-induced alopecia which permit the predictive testing of compounds having human therapeutic efficacy are well-known. Sawada, et al., "Cyclosporin A Stimulates Hair Growth in Nude Mice", *Laboratory Investigation*, 56(6):684 (1987); Holland, "Animal Models of Alopecia", *Clin. Dermatol*, 6:159:162 (1988); Hussein, "Protection from Chemotherapy-induced Alopecia in a Rat Model", *Science*, 249:1564-1566 (1990); and Hussein, et al., "Interleukin 1 Protects against 1-B-D-Arabinofuranosyulcytosine-induced Alopecia in the Newborn Rat Animal Model", *Cancer Research*, 51:3329-3330 (1991).

Male-pattern baldness is prevalent. The progressive loss of hair in men and women is a serious cosmetic problem. According to certain embodiments, this condition may be treated using FGF-like polypeptide either systemically, or topically if the drug could be applied

and absorbed through the scalp, or by spray injection into the scalp using an air gun or similar technologies. A standard in vivo model of male-pattern baldness which permits the predictive testing of compounds having human therapeutic efficacy is well-known. Uno, "The Stumptailed Macaque as a Model for Baldness:effects of Minoxidil", *International Journal of Cosmetic Science*, 8:63-71 (1986).

Gastric ulcers, although treatable by H2 antagonists, cause significant morbidity and a recurrence rate, and heal by scar formation of the mucosal lining. The ability to regenerate glandular mucosa more rapidly would offer a significant therapeutic improvement in the treatment of gastric ulcers. According to certain embodiments, FGF-like polypeptide may be useful for such treatment. Standard in vivo models of gastric ulcers which permit the predictive testing of compounds having human therapeutic efficacy are well-known. Tarnawski, et al., "Indomethacin Impairs Quality of Experimental Gastric Ulcer Healing: A Quantitative Histological and Ultrastructural Analysis", In:*Mechanisms of Injury, Protection and Repair of the Upper Gastrointestinal Tract*, (eds) Garner and O'Brien, Wiley & Sons (1991); and Brodie, "Experimental Peptic Ulcer", *Gastroenterology*, 55:25 (1968).

Duodenal ulcers, like gastric ulcers, are treatable, but the development of a therapeutic agent to more fully and more rapidly regenerate the mucosal lining of the duodenum would be an important advance. In addition, a therapeutic agent to regeneratively heal these ulcers and decrease their recurrence would be of benefit. According to certain embodiments, FGF-like polypeptide may be useful for such treatment. Standard in vivo models of duodenal ulcers which permit the predictive testing of

compounds having human therapeutic efficacy are well-known. Berg, et al., "Duodenal ulcers produced on a diet deficient in pantothenic acid", *Proc. Soc. Exp. Biol. Med.*, 7:374-376 (1949); Szabo and Pihan, "Development and Significance of Cysteamine and Propionitrile Models of Duodenal Ulcer", *Chronobiol. Int.*, 6:31-42 (1987); and Robert, et al., "Production of Secretatogues of Duodenal Ulcers in the Rat", *Gastroenterology*, 59:95-102 (1970).

Erosions of the stomach and esophagus, like erosive gastritis, esophagitis, or esophageal reflux, are treatable but the development of a therapeutic agent to more fully and rapidly regenerate the mucosal lining of the stomach and esophagus would be an important advance. In addition, a therapeutic agent to regeneratively heal these erosions and decrease their recurrence would be of benefit. According to certain embodiments, FGF-like polypeptide may be useful for such treatment. Standard in vivo models of erosion of the stomach and esophagus, like erosive gastritis, esophagitis, or esophageal reflux, which permit the predictive testing of compounds having human therapeutic efficacy are well-known. Geisinger, et al, "The histologic development of acid-induced esophagitis in the cat", *Mod-Pathol.*, 3(5):619-624 (1990); Carlborg, et al., "Tetracycline induced esophageal ulcers. A clinical and experimental study", *Laryngoscope*, 93(2):184-187(1983); Carlborg, et al., "Esophageal lesions caused by orally administered drugs. An experimental study in the cat", Eur-Surg-Ethanol on esophageal motility in cats, *Alcohol-Clin-Exp-Res.*, 15(1):116-121 (1991), and Katz, et al., "Acid-induced esophophagitis in cats is prevented by sucralfate but not synthetic prostaglandin E.", *Dig-Dis-Sci.*, 33(2):217-224 (1988).

Upper and lower gastrointestinal toxicity is a limiting factor in radiation and chemotherapy treatment regimes. According to certain embodiments, pretreatment with FGF-like polypeptide may have a cytoprotective effect on the oral, esophageal, stomach, small intestinal, colonic, and/or rectal mucosa. According to certain embodiments, this may allow increased dosages of such therapies while reducing potential side effects of upper and lower gastrointestinal toxicity. Standard in vivo models of radiation-induced upper and lower gastrointestinal toxicity which permit the predictive testing of compounds having human therapeutic efficacy are well-known. Withers and Elkind, "Microcolony Survival Assay for Cells of Mouse Intestinal Mucosa Exposed to Radiation", *Int. J. Radiat.*, 17(3):261-267 (1970). Standard in vivo models of chemotherapy-induced upper and lower gastrointestinal toxicity which are predictive of human therapeutic efficacy are well-known. Sonis, et al., "An Animal Model for Mucositis Induced by Cancer Chemotherapy, Oral Surg.", *Oral Med. Oral Pathol.*, 69:437-431 (1990); and Moore, "Clonogenic Response of Cells of Murine Intestinal Crypts to 12 Cytotoxic Drugs", *Cancer Chemotherapy Pharmacol.*, 15:11-15 (1985).

Inflammatory bowel diseases, such a Crohn's disease (typically affecting primarily the small intestine) and ulcerative colitis (typically affecting primarily the large bowel), are chronic diseases which result in the destruction of the mucosal surface, inflammation, scar and adhesion formation during repair, and significant morbidity to the affected individuals. A therapeutic to stimulate resurfacing of the mucosal surface, resulting in faster healing, may be of benefit in controlling progression of disease. According to certain embodiments, FGF-like polypeptide may be useful for such treatment. Standard in vivo models of inflammatory bowel d'seas

which permit the predictive testing of compounds having human therapeutic efficacy are well-known. Morris, et al., "Hapten-induced Models of Chronic Inflammation and Ulceration in the Rat Colon", *Gastroenterology*, 96:795-803 (1989); Rachmilewitz, et al., "Inflammatory Mediators of Experimental Colitis in Rats", *Gastroenterology*, 97:326-327 (1989); Allgayer, et al., "Treatment with 16,16'-dimethyl-prostaglandin E2 before and after induction of colitis with trinitrobenzenesulfonic acid in Rats", *Gastroenterology*, 96:1290-1300 (1989); "Review:Experimental Colitis in Animal Models", *Scand. J. Gastroenterol*, 27:529-537 (1992).

Hyaline membrane disease of premature infants results in the absence of surfactant production by type II pneumocytes within the lung, resulting in the collapse of the alveoli. Hyaline membrane disease may have both acute and chronic phases. The acute phase of hyaline membrane disease (Infant Respiratory Distress Syndrome--IRDS) may be treated with mechanical ventilation and treatment with 80-100% concentrations of supplemental oxygen and by administration of an exogenous surfactant. Those patients undergoing a prolonged course of treatment may develop the chronic disease phase of hyaline membrane disease (bronchopulmonary dysplasia--BPD). While the surfactants have greatly reduced the mortality associated with IRDS, the morbidity associated with BPD remains high. It would be useful to have effective treatments to accelerate maturation of the lung and secretion of surfactant in neonates to reduce the incidence of BPD. Although corticosteroids can accelerate maturation and secretion in fetuses twenty-eight weeks old and beyond to a large extent, it would be useful to have treatment for younger fetuses. The history of BPD suggests that improvements in treatment of IRDS will be matched by mechanical ventilation of even smaller

prematurely-born infants and a subsequent increase in the incidence of BPD in these smaller infants. A therapeutic agent that would induce proliferation and differentiation of type II pneumocytes would be of considerable benefit in the treatment of this disease. According to certain embodiments, FGF-like polypeptide may be useful for such treatment. Standard in vivo models of IRDS which permit the predictive testing of compounds having human therapeutic efficacy are well-known. Seider, et al., "Effects of antenatal thyrotropin-releasing hormone, antenatal corticosteroids, and postnatal ventilation on surfactant mobilization in premature rabbits", Am. *J. Obstet. Gynec.*, 166:1551-1559 (1992); Ikegami, et al., "Corticosteroid and thyrotropin-releasing hormone effects on preterm sheep lung function", *J. Appl. Physiol.*, 70:2268-2278 (1991). Standard in vivo models of BPD which permit the predictive testing of compounds having human therapeutic efficacy are well-known. Yuh-Chin, et al., "Natural surfactant and hyperoxide lung injury in primates I. Physiology and biochemistry", *J. Appl. Physiol*. 76:991-1001 (1994); and Galan, et al., "Surfactant replacement therapy in utero for prevention of hyaline membrane disease in the preterm baboon", *Am. J. Obstet. Gynecol.*, 169:817-824 (1993).

Smoke inhalation is a significant cause of morbidity and mortality in the week following a burn injury, due to necrosis of the bronchiolar epithelium and the alveoli. A growth factor that could stimulate proliferation and differentiation of these structures, and induce their repair and regeneration, would be of benefit in treating inhalation injuries. According to certain embodiments, FGF-like polypeptide may be useful for such treatment. A standard in vivo model of smoke inhalation which permits the predictive testing of compounds having human therapeutic efficacy is well-known. Hubbard, et al.,

"Smoke inhalation injury in sheep", *Am. J. Pathol.*, 133:660-663 (1988).

Emphysema results from the progressive loss of alveoli. A growth factor that could stimulate regrowth or, which is cytoprotective for remaining alveoli may be of therapeutic benefit. According to certain embodiments, FGF-like polypeptide may be useful for such treatment. A standard in vivo model of emphysema which permits the predictive testing of compounds having human therapeutic efficacy is well-known. "Induction of emphysema and bronchial mucus cell hyperplasia by intratracheal instillation of lipopolysaccharide in the hamster." *J. Pathol.*, 167:349-56 (1992).

Hepatic cirrhosis, secondary to viral hepatitis and chronic alcohol ingestion, is a significant cause of morbidity and mortality. Cytoprotection, proliferation, and differentiation of hepatocytes to increase liver function may be of benefit to slow or prevent the development of cirrhosis. According to certain embodiments, FGF-like polypeptide may be useful for such treatment. A standard in vivo model of hepatic cirrhosis which permits the predictive testing of compounds having human therapeutic efficacy is well-known. Tomaszewski, et al., "The production of hepatic cirrhosis in rats", *J. Appl. Toxicol.*, 11:229-231 (1991).

Fulminant liver failure is a life-threatening condition which occurs with endstage cirrhosis. An agent that could induce proliferation of remaining hepatocytes may be of direct benefit to this disease. According to certain embodiments, FGF-like polypeptide may be useful for such treatment. Standard in vivo models of fulminant liver failure which permit the predictive testing of compounds having human therapeutic efficacy are well-

known. Mitchell, et al., "Acetaminophen-induced hepatic necrosis I. Role of drug metabolism", *J. Pharmcol. Exp. Ther.*, 187:185-194 (1973); and Thakore and Mehendale, "Role of hepatocellular regeneration in CC14 autoprotection", *Toxicologic Pathol.* 19:47-58 (1991).

Acute viral hepatitis is frequently subclinical and self-limiting. However, in a minority of patients, severe liver damage can result over several weeks. A cytoprotective agent may be of use in preventing hepatocellular degeneration. According to certain embodiments, FGF-like polypeptide may be useful for such treatment.

It would be useful to treat thymic epithelial atrophy. This condition may occur in myasthenia gravis, HIV-1 infection, and thymic involution. Haynes, et al., "The human thymus. A chimeric organ comprised of central and peripheral lymphoid components.", *Immunol Res* 1998;18(2):61-78. Therefore, an agent that stimulates growth of thymic epithelial cells may be beneficial in treating these conditions. According to certain embodiments, FGF-like polypeptide may be useful for such treatment.

One skilled in the art would recognize various therapeutic uses for FGF-like polypeptide based on its effect on epithelial cells. These therapeutic uses include, but are not limited to, the stimulation of wound healing, the reduction of scarring, the treatment of Adult Respiratory Distress Syndrome, the treatment of pressure ulcers, and xerostomia. In addition, antagonists against FGF-like polypeptide may be used in therapy of conditions of epithelial hyperplasia such as, but not limited to, carcinomas. Various therapeutic uses for KGF that may apply to FGF-like polypeptide according

to certain embodiments are discussed, e.g., in U.S. Patent Nos. 5,858,977, 5,965,530, and 5,824,643 (herein incorporated by reference for any purpose).

Accordingly, certain embodiments of the present invention encompass the use of FGF-like polypeptide therapeutically (or where appropriate, prophylactically) to treat conditions such as, but not limited to, the above mentioned conditions, as well as pharmaceutical preparations containing FGF-like polypeptide in suitable, therapeutically effective amounts. The actual dosing and formulation may be determined by one skilled in the art using techniques such as those discussed above. Other factors that may play a role in dosing, according to certain embodiments, include the severity of the wound, the condition of the patient, the age of the patient and any collateral injuries or medical ailments possessed by the patient. According to certain embodiments, the amount of active ingredient may be in the range of about 1 $\mu g/cm^2$ to 5 mg/cm^2.

Additional Uses of FGF-like Nucleic Acids and Polypeptides

Nucleic acid molecules of the present invention (including those that do not themselves encode biologically active polypeptides) may be used to map the locations of the FGF-like gene and related genes on chromosomes. Mapping may be done by techniques known in the art, such as PCR amplification and *in situ* hybridization. For example, the murine FGF-like nucleotide sequence of the present invention was used to map the human ortholog of the present invention to human chromosome 19p13.3.

FGF-like nucleic acid molecules (including those that do not themselves encode biologically active

polypeptides), may be useful as hybridization probes in diagnostic assays to test, either qualitatively or quantitatively, for the presence of an FGF-like DNA or corresponding RNA in mammalian tissue or bodily fluid samples.

The FGF-like polypeptides may be used (simultaneously or sequentially) in combination with one or more cytokines, growth factors, antibiotics, anti-inflammatories, and/or chemotherapeutic agents as is appropriate for the indication being treated.

Other methods may also be employed where it is desirable to inhibit the activity of one or more FGF-like polypeptides. Such inhibition may be effected by nucleic acid molecules which are complementary to and hybridize to expression control sequences (triple helix formation) or to FGF-like mRNA. For example, antisense DNA or RNA molecules, which have a sequence that is complementary to at least a portion of the selected FGF-like gene(s) can be introduced into the cell. Anti-sense probes may be designed by available techniques using the sequence of FGF-like polypeptide disclosed herein. Typically, each such antisense molecule will be complementary to the start site (5' end) of each selected FGF-like gene. When the antisense molecule then hybridizes to the corresponding FGF-like mRNA, translation of this mRNA is prevented or reduced. Anti-sense inhibitors provide information relating to the decrease or absence of an FGF-like polypeptide in a cell or organism.

Alternatively, gene therapy may be employed to create a dominant-negative inhibitor of one or more FGF-like polypeptides. In this situation, the DNA encoding a mutant polypeptide of each selected FGF-like polypeptide can be prepared and introduced into the cells of a patient using either viral or non-viral methods as

described herein. Each such mutant is typically designed to compete with endogenous polypeptide in its biological role.

In addition, an FGF-like polypeptide, whether biologically active or not, may be used as an immunogen, that is, the polypeptide contains at least one epitope to which antibodies may be raised. Selective binding agents that bind to an FGF-like polypeptide (as described herein) may be used for *in vivo* and *in vitro* diagnostic purposes, including, but not limited to, use in labeled form to detect the presence of FGF-like polypeptide in a body fluid or cell sample. The antibodies may also be used to prevent, treat, or diagnose a number of diseases and disorders, including those recited herein. The antibodies may bind to an FGF-like polypeptide so as to diminish or block at least one activity characteristic of an FGF-like polypeptide, or may bind to a polypeptide to increase at least one activity characteristic of an FGF-like polypeptide (including by increasing the pharmacokinetics of the FGF-like polypeptide).

The following examples are intended for illustration purposes only, and should not be construed as limiting the scope of the invention in any way.

Example 1: DNA Encoding Human FGF-like polypeptide

Materials and methods for cDNA cloning and analysis are described in Sambrook *et al., supra*.

Polymerase chain reactions (PCR) are generally performed using a Perkin-Elmer 9600 thermocycler and employing a commercially available PCR reaction mixture (Boehringer Mannheim, Indianapolis, IN) and primer concentrations specified by the manufacturer. In general, 25-50 µl reactions containing template nucleic

acid molecules are incubated at 94°C, followed by 20-40 cycles of 94°C for five seconds, 50-60°C for five seconds, and 72°C for 3-5 minutes. Reactions are then analyzed by gel electrophoresis as described in Sambrook *et al.*, *supra*.

Human placenta poly A+ RNA (1 µg) was incubated for 60 min at 37°C in a reaction mixture (20 µl) containing 300 units of Moloney murine leukemia virus reverse transcriptase, 15 units of human placenta RNase inhibitor and 0.5 µg of a random hexadeoxynucleotide primer. Human FGF-like cDNA including the entire coding region was amplified by polymerase chain reaction (PCR) (30 cycles) in a reaction mixture (25 µl) containing 1 µl of cDNA, 0.05 unit/µl Ex Taq DNA polymerase, 10% dimethyl sulfoxide (DMSO) and 0.4 pmol/µl of each of a sense primer (5'-cgacgagcgcgcagcgaac-3')(SEQ ID NO: 25) and an antisense primer (5'-ctctcagggcctcaggaga-3') (SEQ ID NO: 26)(the "PCR solution"). Human FGF-like cDNA was further amplified by PCR (30 cycles) in a reaction mixture (25 µl) containing 1 µl of the PCR solution, 0.05 unit/µl Ex Taq DNA polymerase, 10% dimethyl sulfoxide (DMSO) and 0.4 pmol/µl of each of a sense primer (5'-aaccgggtgccgggtcatg-3') (SEQ ID NO: 27) and an antisense primer (5'-gcctcaggagaccaggac-3') (SEQ ID NO: 28). The amplified FGF-like cDNA was cloned into the pGEM-T DNA vector and the nucleotide sequence was determined with a DNA sequencer.

The nucleotide sequence of human FGF-like polypeptide is shown in Figure 1 (SEQ ID NO: 1). When the predicted amino acid sequence, also shown in Figure 1 (SEQ ID NO: 2; mature form SEQ ID NO: 3), is analyzed by the method of Nielsen et al., a 22 amino acid cleavable signal sequence is identified at its amino terminus. Thus, the amino-terminus of the mature form of the FGF-like polypeptide of the present invention is threonine$_{23}$

of SEQ ID NO: 2. The underlined sequence in Figure 1, SEQ ID NO: 2, is the cleavable signal sequence of the precursor; the remaining sequence, corresponding to SEQ ID NO: 3, represents the mature form of the FGF-like polypeptide. The nucleotide and amino acid sequences for the murine FGF-like polypeptide is shown in Figure 2.

As would be expected for a member of the FGF family of growth factors, the FGF-like polypeptide precursor of the present invention contains a cleavable signal sequence indicating that it is secreted from the cell. The mature form of the FGF-like polypeptide of the present invention, with a predicted molecular weight of 17.2 kilodaltons (kDa), does not contain N-linked oligosaccharides as it lacks the Asn-X-Ser/Thr consensus sequence. The predicted molecular weight of the FGF-like polypeptide precursor is 19.7 kDa.

Example 2: Tissue Expression

Tissue expression patterns of FGF-like mRNA in mouse tissue was determined by Northern blot analysis, according to the manufacturer's instructions (Multiple Choice™, OriGene Technologies, Inc., Rockville, MD), using a ^{32}P-labeled murine FGF-like cRNA.

Approximately 2 µg of mouse poly-A+ RNA isolated from stomach, small intestine, skeletal muscle, lung, testis, skin, brain, heart, kidney, spleen, thymus, and liver were electrophoresed in a 1% denaturing formaldehyde agarose gel. RNA in the gel was transferred to a positively charged nylon membrane and then crosslinked by UV irradiation.

These blots were prehybridized in 5X SSPE, 50% formamide, 5X Denhardt's solution, 0.2% SDS, 5% dextran sulfate, and 100 µg/ml denatured, sheared salmon sperm

DNA (hybridization buffer) for 2-4 hours at 42°C. The blots were hybridized overnight at 42°C in hybridization buffer containing ^{32}P-labeled murine FGF-like cDNA probe with a specific activity of 2X10^6 cpm/ml. The ^{32}P-labeled cDNA probe was prepared using the Ready-To-Go™ DNA labeling beads following the manufacturer's instructions (Pharmacia Biotech).

The hybridized blots were washed three times in 2X salt sodium citrate (SSC), 0.1% SDS for 5 minutes per wash at room temperature, then twice in 0.25X SSC, 0.1% SDS for 30 minutes at 65°C. X-ray film was exposed using these blots at -70°C with an intensifying screen. The exposed film was developed to determine tissue expression of the FGF-like polypeptide. Hybridization occurred only with mRNA from the skin sample.

Example 3: Production of FGF-like Polypeptides

A. Baculovirus Expressed FGF-like Polypeptide

Mouse FGF-like cDNA with a DNA fragment (75 bp) encoding an E tag (GAPVPYPDPLEPR)(SEQ ID NO: 29) and a 6X His tag (HHHHHH) (SEQ ID NO: 30) at the 3 -terminus of the coding region was constructed in a transfer vector DNA, pBacPAK9. Recombinant baculovirus containing the cDNA with the tag sequences was obtained by co-transfection of Spedoptera Sf9 insect cells with the recombinant pBacPAK9 and a Bsu36 I-digested expression vector, BacPAK6. Sf9 insect cells were infected with the resultant recombinant baculovirus and incubated at 27°C for 24 hours in TC-100 insect medium supplemented with 10% fetal bovine serum (supplemented TC-100 medium). After the infection, the cells were cultured at 27°C for 60 hours in supplemented TC-100 medium.

The expression of FGF-like polypeptides was monitored by Western blot analysis. Culture media or Sf9 cell lysates were electrophoresed on 12.5% SDS-PAGE gels under reducing conditions and transferred to a nitrocellulose membrane. The membrane was incubated with radiolabeled anti-E tag antibodies. The membrane was washed, dried and placed on x-ray film. The presence of expressed E tag-FGF-like polypeptides was determined by the presence or absence of a band on the developed film.

B. Bacterial Expression

PCR is used to amplify template DNA sequences encoding an FGF-like polypeptide using primers corresponding to the 5' and 3'ends of the sequence, e.g., (5'-aaccgggtgccgggtcatg-3') (SEQ ID NO: 27) and (5'-gcctcaggagaccaggac-3') (SEQ ID NO: 28). The amplified DNA products may be modified to contain restriction enzyme sites to allow for insertion into expression vectors. PCR products are gel purified and inserted into expression vectors using standard recombinant DNA methodology. An exemplary vector, such as pAMG21 (ATCC No. 98113) containing the lux promoter and a gene encoding kanamycin resistance is digested with BamHI and NdeI for directional cloning of inserted DNA. The ligated mixture is transformed into an *E. coli* host strain by electroporation and transformants are selected for kanamycin resistance. Plasmid DNA from selected colonies is isolated and subjected to DNA sequencing to confirm the presence of the insert.

Transformed host cells are incubated in 2xYT medium containing 30 µg/ml kanamycin at 30°C prior to induction. Gene expression is induced by the addition of N-(3-oxohexanoyl)-dl-homoserine lactone to a final concentration of 30 ng/ml followed by incubation at

either 30°C or 37°C for six hours. The expression of FGF-like polypeptide is evaluated by centrifugation of the culture, resuspension and lysis of the bacterial pellets, and analysis of host cell proteins by SDS-polyacrylamide gel electrophoresis.

Inclusion bodies containing FGF-like polypeptide are purified as follows. Bacterial cells are pelleted by centrifugation and resuspended in water. The cell suspension is lysed by sonication and pelleted by centrifugation at 195,000xg for 5 to 10 minutes. The supernatant is discarded, and the pellet is washed and transferred to a homogenizer. The pellet is homogenized in 5 ml of a Percoll solution (75% liquid Percoll. 0.15M NaCl) until uniformly suspended and then diluted and centrifuged at 21,600xg for 30 minutes. Gradient fractions containing the inclusion bodies are recovered and pooled. The isolated inclusion bodies are analyzed by SDS-PAGE.

A single band on an SDS polyacrylamide gel corresponding to *E. coli*-produced FGF-like polypeptide is excised from the gel, and the N-terminal amino acid sequence is determined essentially as described by Matsudaira *et al.*, *J. Biol. Chem.*, 262:10-35 (1987).

C. Mammalian Cell Production

PCR is used to amplify template DNA sequences encoding an FGF-like polypeptide using primers corresponding to the 5' and 3' ends of the sequence, e.g., (5'-aaccgggtgccgggtcatg-3') (SEQ ID NO: 27) and (5'-gcctcaggagaccaggac-3') (SEQ ID NO: 28). The amplified DNA products may be modified to contain restriction enzyme sites to allow for insertion into expression vectors. PCR products are gel purified and

inserted into expression vectors using standard recombinant DNA methodology. An exemplary expression vector, pCEP4 (Invitrogen, Carlsbad, CA), which contains an Epstein-Barr virus origin of replication, may be used for the expression of FGF-like in 293-EBNA-1 (Epstein-Barr virus nuclear antigen) cells. Amplified and gel purified PCR products are ligated into pCEP4 vector and lipofected into 293-EBNA cells. The transfected cells are selected in 100 µg/ml hygromycin and the resulting drug-resistant cultures are grown to confluence. The cells are then cultured in serum-free media for 72 hours. The conditioned media is removed and, FGF-like polypeptide expression is analyzed by SDS-PAGE.

FGF-like polypeptide expression may be detected by silver staining. Alternatively, FGF-like polypeptide is produced as a fusion protein with an epitope tag, such as an IgG constant domain or a FLAG epitope, which may be detected by Western blot analysis using antibodies to the tag peptide.

FGF-like polypeptides may be excised from an SDS-polyacrylamide gel, or FGF-like fusion proteins are purified by affinity chromatography to the epitope tag, and subjected to N-terminal amino acid sequence analysis as described herein.

Example 4: Production of anti-FGF-like polypeptide antibodies

Antibodies to FGF-like polypeptides may be obtained by immunization with purified protein or with FGF-like peptides produced by biological or chemical synthesis. Suitable procedures for generating antibodies include

those described in Hudson and Hay, *Practical Immunology, 2nd Edition*, Blackwell Scientific Publications (1980).

In one procedure for the production of antibodies, animals (typically mice or rabbits) are injected with an FGF-like antigen (such as an FGF-like polypeptide), and those with sufficient serum titer levels as determined by ELISA are selected for hybridoma production. Spleens of immunized animals are collected and prepared as single cell suspensions from which splenocytes are recovered. The splenocytes are fused to mouse myeloma cells (such as Sp2/0-Ag14 cells; ATCC no. CRL-1581), allowed to incubate in DMEM with 200 U/ml penicillin, 200 µg/ml streptomycin sulfate, and 4 mM glutamine, then incubated in HAT selection medium (Hypoxanthine; Aminopterin; Thymidine). After selection, the tissue culture supernatants are taken from each fusion well and tested for anti-FGF-like antibody production by ELISA.

Alternative procedures for obtaining anti-FGF-like antibodies may also be employed, such as the immunization of transgenic mice harboring human Ig loci for the production of human antibodies, and the screening of synthetic antibody libraries, such as those generated by mutagenesis of an antibody variable domain.

Example 5: Biological Activity Assays for FGF and FGF-like polypeptides

A. Mitogenic Activity Assay

NIH/3T3 or fetal rat skin keratinizing (FRSK) epidermal cells are seeded at 1000 cells/well in a 96-well tissue culture plate in Dulbecco's modified Eagle's medium containing 10% calf serum or Ham's F-12 medium containing 10% fetal bovine serum, respectively, and cultured for 4-5 days. When the cells are approximately

80% confluent, they are washed twice with phosphate-buffered saline and then cultured for an additional 24 h. Cultures are then supplemented with FGF or FGF-like polypeptide. [^{3}H]Thymidine is added to each well (7.4 kBq/well) 17 h after supplementation; 4 h later, the cells are lysed with 2 N NaOH and harvested onto filters using a Skatron microcell harvester. Filters are dried, and the radioactivity of each filter is measured in a liquid scintillation counter. (H. Emoto et al., J. Biol. Chem. 272 (1997) 23224- 23227).

B. Neurite Outgrowth Assay.

PC12 cells are seeded in 24-well culture plates coated with poly-L-lysine in Dulbecco's modified Eagle's medium, supplemented with 10% fetal calf serum and 5% horse serum and incubated at 37°C in 5% CO_2 with humidity. After 48 hours, the cultures are supplemented with FGF or FGF-like polypeptide and incubated for an additional 72 hours. Outgrowth of neurites from the cells is monitored using a phase-contrast microscope. (Ohbayashi et al., J. Biol. Chem. 273 (1998) 18161-18164).

Example 6: Pathologic Analysis of Transgenic Mice Overexpressing Murine FGF-like polypeptide

A. Preparation of Transgenic Mice

The coding region of a murine cDNA encoding FGF-like polypeptide with an altered Kozak sequence, CCACC, immediately upstream of the initiating ATG, was obtained by PCR with transgene specific primers 5'CTA TAA GCT TCC ACC ATG CGC AGC CGC CTC TGG3' and 5'CTC TGG ATC CGG CCC TTC AAG ACG AGA C3'. The coding region of the DNA had the sequence from position 140 (commencing with the codon ATG) to position 625 (just prior to TGA)of SEQ ID NO: 34. The PCR amplification product was ligated into a beta

actin-specific expression vector (as described in Klebig et al, *Ectopic expression of the agouti gene in transgenic mice causes obesity, features of type II diabetes, and yellow fur*, Proc. Natl. Acad. Sci., vol 92, p.4728-32 (1995)). The expression vector includes a 3.4 kb DNA fragment that contains the human beta actin promoter and a 837 bp human beta actin intron. A SV40 polyadenylation signal is located downstream of the cDNA insert sites. The integrity of the cDNA is verified by sequencing. The resulting plasmid labeled TA00-005 was transfected into bacteria to obtain more plasmid.

The plasmids from the bacteria were purified, and the transgene insert (which includes human beta actin promoter, the human beta actin intron, the PCR amplification product including the coding region of DNA encoding the FGF-like molecule and the altered Kozak sequence, and the SV40 polyadenylation signal) was isolated from the plasmid with restriction enzymes. That transgene insert was microinjected into single-cell embryos from BDF1 x BDF1-bred mice (BDF1 mice are available from Charles River Laboratories, Wilmington, Mass.) as described in Brinster et al., Proc. Natl. Acad. Sci., 82:4438-4442 (1985). Embryos were cultured overnight in a 37°C and 5% CO_2 incubator and 15 to 20 2-cell embryos were transferred to the oviducts of 25 pseudopregnant CD1 female mice and 16 litters were born. Transgenic offspring were identified by PCR screening with primers 5'GAT GAG TTT GGA CAA ACC ACA3' and 5' CCG GAT CAT AAT CAG CCA TAC3' that amplify a 220 bp fragment of the SV40PA from DNA prepared from ear biopsies.

B. RNA Analysis

At 8-10 weeks of age, 10 potentially transgenic and five nontransgenic littermates were necropsied. Muscle

samples from these mice are flash frozen in liquid nitrogen at the time of necropsy. RNAs were isolated from each sample using Trizol (Life Tecnologies, Inc.). Northern Blot was generated by running 10 µg of RNA in 1X RNA Loading Dye (Sigma) on a 1% formaldehyde-agarose gel. The gel was denatured in 50 mM NAOH and 150 mM NaCl, neutralized in 0.1M Tris-HCl, pH 7.0 and 150 mM NaCl and blotted onto a Duralon membrane according to the manufacturer (Stratagene). The Northern Blot was probed with a ^{32}P-labeled cDNA that was generated by the Rediprime System (Amersham), using the Express Hyb Solution (Clontech) and then washed according to the manufacturer. The hybridized blot was exposed to film (Kodak) for 72 hours at -80°C and then developed. Of the ten potential transgenic mice, seven (two males and five females) were shown to actually be transgenic based on the Northern Blot results.

Subsequently, an additional four transgenic mice (all female) were sacrificed about a month later due to their moribund condition and were shown to be transgenic based on Northern Blot analysis using the procedures discussed above.

C. Necropsy

The first seven transgenic mice discussed above in section (B) (two males and five females at 6-8 weeks old), which were transgenic for murine FGF-like polypeptide targeted for ubiquitous overexpression via a β-actin promoter, as well as the five, 6-8 week old, non-transgenic BDF1 littermate mice (three males and two females) were pathologically analyzed for a potential FGF-like polypeptide phenotype. The additional four transgenic mice discussed in section (B) above, which were FGF-like polypeptide transgenic mice (all female), were necropsied at a later date due to their moribund

condition, and were also pathologically analyzed. Mice #'s 15, 19, 21, 41 and 109 were strongly positive for muscle expression of FGF-like polypeptide mRNA, while mice 1 and 3 were weakly positive. The four additional female FGF-like polypeptide transgenic mice analyzed at a later date, #'s 83, 91, 110 and 118, were all moderately to strongly positive for muscle FGF-like polypeptide mRNA expression. Mice #'s 14, 18, 81, 82 and 120 were negative mice, i.e. they were non-transgenic. One hour prior to necropsy, mice were injected intraperitoneally with 50 mg/kg of bromo-deoxyuridine (BrdU). At necropsy, liver, spleen, kidney, heart, and thymus were weighed. Sections of liver, spleen, lung, brain, heart, kidney, adrenal, thymus, stomach, small intestine, pancreas, cecum, colon, mesenteric lymph node, skin, mammary gland, trachea, esophagus, thyroid, parathyroid, salivary gland, urinary bladder, ovary or testis, uterus or seminal vesicle, skeletal muscle, bone, and bone marrow, as well as cutaneous papillomas from several of the FGF-like polypeptide transgenic mice were harvested for histologic analysis.

D. Histology

Sections of liver, spleen, lung, brain, heart, kidney, adrenal, thymus, stomach, small intestine, pancreas, cecum, colon, mesenteric lymph node, skin, mammary gland, trachea, esophagus, thyroid, parathyroid, salivary gland, urinary bladder, ovary or testis, uterus or seminal vesicle, skeletal muscle, bone, and bone marrow from the FGF-like polypeptide transgenic and non-transgenic mice were fixed overnight in 10% neutral buffered zinc formalin (Anatech, Battle Creek, Michigan), paraffin embedded, sectioned at 3 µm, and stained with hematoxylin and eosin (H&E) (see below for

routine histologic examination). In addition, 4 µm thick sections were prepared after the fixing and paraffin embedding discussed above, and those sections were immunostained for BrdU and examined (see below).

E. BrdU Immunohistochemistry

Immunohistochemical staining for BrdU was done on the 4 µm thick paraffin embedded sections using an automated DPC Mark 5 Histochemical Staining System (Diagnostic Products Corp, Randolph, NJ). Deparaffinized tissue sections were digested with 0.1% protease and then treated with 2N hydrochloric acid. Sections were blocked with CAS BLOCK (Zymed Laboratories, San Francisco, CA), incubated with a rat anti-BrdU monoclonal antibody (Accurate Chemical and Scientific, Westbury, NY). The primary antibody was detected with a biotinylated rabbit anti-rat immunoglobulin polyclonal antibody (Dako, Carpinteria, CA). Sections were then quenched with 3% hydrogen peroxide, and reacted with an avidin-biotin complex tertiary (Vector Laboratories). The staining reaction was visualized with diaminobenzidine (DAB, Dako Carpinteria, CA) and sections were counterstained with hematoxylin.

F. Gross Pathology Findings

Significant gross findings in the FGF-like polypeptide transgenic mice fell into four major categories. First, two of the FGF-like polypeptide transgenic mice from the first necropsy (#'s 19 and 21) and all of the FGF-like polypeptide transgenic mice from the second necropsy (#'s 83, 91, 110 and 118) had one or more multiple cutaneous papillomatous growths. The second significant gross finding was that the same two FGF-like polypeptide transgenic mice with the papillomas (#'s 19

and 21) as well as three of the four FGF-like polypeptide transgenic mice from the second necropsy also exhibited marked thymic enlargement (mean of 1.92 ± 1.16 SD % of body weight vs. 0.22 ± 0.08 SD % of body weight in non-transgenic control mice, p=0.012). Third, three of the transgenic mice (#15 from the first necropsy and #s 83 and 118 from the second necropsy) exhibited moderate to marked splenomegaly (mean of 1.69 % of body weight ± 1.21 SD % of body weight vs. 0.32 ± 0.06 SD % of body weight in non-transgenic control mice, not statistically significant). Lastly, one FGF-like polypeptide transgenic mouse (#15 from the first necropsy) had marked hepatomegaly (8.18% of its body weight). The raw organ weight data is shown in Table 3.

Table 3 Raw Organ Weight Data for FGF-like polypeptide Transgenic Mice vs. Non-Transgenic Mice

First Necropsy

Group	Sex	TBW	Liver	%BW	Spleen	%BW	Heart	%BW	Kidneys	%BW	Thymus	%BW
Non-transgenic												
14	F	26.6	1.455	5.47	0.115	0.43	0.13	0.49	0.41	1.54	0.082	0.31
18	F	25.1	1.209	4.82	0.077	0.31	0.13	0.52	0.387	1.54	0.051	0.20
81	M	26.3	1.231	4.68	0.073	0.28	0.124	0.47	0.434	1.65	0.047	0.18
82	M	19.4	0.70	3.61	0.055	0.28	0.111	0.57	0.31	1.6	0.059	0.30
120	M	33.4	1.783	5.34	0.104	0.31	0.152	0.46	0.572	1.71	0.042	0.13
Mean				4.78		0.32		0.5		1.61		0.22
St. Dev.				0.74		0.06		0.04		0.07		0.08
FGF-like polypeptide Transgenic												
1	F	21.1	1.028	4.87	0.075	0.36	0.117	0.55	0.359	1.7	0.073	0.35
3	F	26.1	1.09	4.18	0.084	0.32	0.132	0.51	0.342	1.31	0.073	0.28
15	F	27.8	2.273	8.18	0.207	0.74	0.137	0.49	0.424	1.53	0.069	0.25
19	F	22.8	1.105	4.85	0.131	0.57	0.12	0.53	0.405	1.78	0.173	0.76
21	F	30.1	1.467	4.87	0.121	0.40	0.145	0.48	0.429	1.43	0.419	1.39
41	M	30.6	1.706	5.58	0.136	0.44	0.166	0.54	0.591	1.93	0.109	0.36
109	M	27.2	1.639	6.03	0.128	0.47	0.179	0.66	0.562	2.07	0.127	0.47
Mean				5.51		0.47		0.54		1.68		0.55
St. Dev.				1.32		0.14		0.06		0.27		0.41

Second Necropsy

Group	Sex	TBW	Liver	%BW	Spleen	%BW	Heart	%BW	Kidneys	%BW	Thymus	%BW
FGF-like polypeptide Transgenic												
110	F	24.1	1.185	4.92	0.081	0.34	0.138	0.57	0.424	1.76	0.07	0.29
91	F	19.7	0.866	4.40	0.109	0.55	0.125	0.64	0.383	1.94	0.274	1.39
118	F	19.6	1.237	6.31	0.598	3.05	0.125	0.64	0.348	1.78	0.451	2.30
83	F	28.5	1.493	5.24	0.362	1.27	0.178	0.63	0.447	1.57	1.065	3.74
Mean				5.22		1.30		0.62		1.76		1.93
St. Dev.				0.81		1.23		0.03		0.15		1.46

G. Histopathologic Findings

H&E and BrdU stained sections of liver, spleen, lung, brain, heart, kidney, adrenal, thymus, stomach, small intestine, pancreas, cecum, colon, mesenteric lymph node, skin, mammary gland, trachea, esophagus, thyroid, parathyroid, salivary gland, urinary bladder, ovary or testis, uterus or seminal vesicle, skeletal muscle, bone, bone marrow, and cutaneous papillomas (when present) were

examined from the 11 FGF-like polypeptide transgenic mice and 5 non-transgenic control littermates. Significant histologic findings in the FGF-like polypeptide transgenic mice fell into four major categories. First, two of the FGF-like polypeptide transgenic mice from the first necropsy (#s 19 and 21) and all four mice from the second necropsy (#s 83, 91, 110 and 118) had one to multiple cutaneous papillomas at various sites, some with significant adnexal (hair follicular or sebaceous glandular) involvement. Second, the same two mice that had cutaneous papillomas in the first necropsy (#s 19 and 21) as well as three of the four mice with papillomas from the second necropsy (#s 83, 91 and 118) also exhibited marked thymic enlargement characterized by thymic cortical expansion with disruption of normal thymic architecture. Third, one of the FGF-like polypeptide transgenic mice in the first necropsy (#15), exhibited marked hepatomegaly with marked hepatocellular hyperplasia and dysplasia characterized by binucleate cells and nuclear atypia. This mouse's liver also had a focus of hepatocellular necrosis and mineralization at the tip of a lobe. Another major finding was papillomatous hyperplasia of the non-glandular squamous stomach in transgenic mouse #118 from the second necropsy. Several of the FGF-like polypeptide transgenic mice (#15 from the first necropsy and #s 83 and 115 from the second necropsy) also exhibited splenomegaly due to organized hyperplasia of both the red pulp and lymphoid follicles.

H. Summary of Phenotypic Findings in Transgenic Mice Overexpressing Murine FGF-like Polypeptide

The FGF-like polypeptide transgenic mice have a variable phenotype, with a consistent finding being

multifocal cutaneous papillomas and/or epidermal/adnexal papillomatous hyperplasia. Another common finding was thymic hyperplasia, with other, more variable findings including hyperplasia of the squamous stomach, hepatocellular hyperplasia and dysplasia, and splenic red and white pulp hyperplasia. It appears that some form of epithelial hyperplasia may contribute to these phenotypic changes, except perhaps the splenomegoly. Epidermis and epidermal adnexa clearly appear to be susceptible to the effects of FGF-like polypeptide overexpression. All of these findings suggest that the FGF-like polypeptide protein plays a role in the development, stimulation and/or repair of multiple epithelial tissues.

While the present invention has been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations that come within the scope of the invention as claimed.

WHAT IS CLAIMED

1. An isolated nucleic acid molecule comprising a nucleotide sequence selected from:

(a) the nucleotide sequence as set forth in SEQ ID NO: 1;

(b) a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NO: 2;

(c) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of (a) or (b), wherein the encoded polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3; and

(d) a nucleotide sequence complementary to any of (a)-(c).

2. An isolated nucleic acid molecule comprising a nucleotide sequence selected from:

(a) a nucleotide sequence encoding a polypeptide that is at least about 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the polypeptide as set forth in SEQ ID NO: 3, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(b) a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence as set forth in SEQ ID NO: 1, wherein the encoded polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(c) a nucleotide sequence of SEQ ID NO: 1; (a); or (b) encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(d) a nucleotide sequence of SEQ ID NO: 1, or (a)-

(c) comprising a fragment of at least about 16 nucleotides;

(e) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(d), wherein the polypeptide encoded by the nucleotide sequence has an activity of the polypeptide as set forth in SEQ ID NO: 3; and

(f) a nucleotide sequence complementary to any of (a)-(c).

3. An isolated nucleic acid molecule comprising a nucleotide sequence selected from:

(a) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 3 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(b) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 3 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(c) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 3 with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(d) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 3 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(e) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NO: 3 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal

truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(f) a nucleotide sequence of (a)-(e) comprising a fragment of at least about 16 nucleotides;

(g) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a)-(f), wherein the polypeptide encoded by the nucleotide sequence has an activity of the polypeptide as set forth in SEQ ID NO: 3; and

(h) a nucleotide sequence complementary to any of (a)-(e).

4. A vector comprising the nucleic acid molecule of Claims 1, 2, or 3.

5. A host cell comprising the vector of Claim 4.

6. The host cell of Claim 5 that is a eukaryotic cell.

7. The host cell of Claim 5 that is a prokaryotic cell.

8. A process of producing an FGF-like polypeptide comprising culturing the host cell of Claim 5 under suitable conditions to express the polypeptide.

9. A process according to claim 8, further comprising isolating the polypeptide from the culture.

10. An FGF-like polypeptide produced by the process of Claim 8.

11. The process of Claim 8, wherein the nucleic acid

molecule comprises promoter DNA other than the promoter DNA for the native FGF-like polypeptide operatively linked to the DNA encoding the FGF-like polypeptide.

12. The isolated nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide that is at least about 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the polypeptide as set forth in SEQ ID NO: 3, wherein the polypeptide encoded by the nucleic acid sequence has an activity of the polypeptide as set forth in SEQ ID NO: 3, and wherein the percent identity is determined using a computer program selected from the group consisting of GAP, BLASTP, BLASTN, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm.

13. A process for determining whether a compound inhibits FGF-like polypeptide activity or production comprising exposing a cell according to Claim 5 to the compound, and measuring FGF-like polypeptide activity or production in said cell.

14. The process of claim 13, wherein the cell is a prokaryotic cell or a eukaryotic cell.

15. An isolated polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 3.

16. An isolated polypeptide comprising the amino acid sequence selected from:

(a) the mature amino acid sequence as set forth in SEQ ID NO: 3;

(b) the mature amino acid sequence as set forth in SEQ ID NO: 3 with an amino-terminal methionine;

(c) an amino acid sequence for an ortholog of SEQ ID

NO: 3, wherein the encoded polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(d) an amino acid sequence that is at least about 70, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the amino acid sequence of SEQ ID NO: 3, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(e) a fragment of the amino acid sequence set forth in SEQ ID NO: 3 comprising at least about 25 amino acid residues, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3; and

(f) an amino acid sequence for an allelic variant or splice variant of either the amino acid sequence as set forth in SEQ ID NO: 2, or at least one of (a)-(d) wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3.

17. An isolated polypeptide comprising the amino acid sequence selected from:

(a) the amino acid sequence as set forth in SEQ ID NO: 3 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(b) the amino acid sequence as set forth in SEQ ID NO: 3 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(c) the amino acid sequence as set forth in SEQ ID NO: 3 with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3;

(d) the amino acid sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of

the polypeptide as set forth in SEQ ID NO: 3; and

(e) the amino acid sequence as set forth in SEQ ID NO: 3, with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3.

18. An isolated polypeptide encoded by the nucleic acid molecule of Claims 1, 2, or 3.

19. The isolated polypeptide according to Claim 16 comprising an amino acid sequence that is at least about 70, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the amino acid sequence of SEQ ID NO: 3, wherein the polypeptide has an activity of the polypeptide as set forth in SEQ ID NO: 3, and wherein the percent identity is determined using a computer program selected from the group consisting of GAP, BLASTP, BLASTN, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm.

20. An antibody produced by immunizing an animal with a peptide comprising an amino acid sequence of SEQ ID NO: 3.

21. An antibody or fragment thereof that specifically binds the polypeptide of Claims 15, 16, or 17.

22. The antibody of Claim 21 that is a monoclonal antibody.

23. A hybridoma that produces a monoclonal antibody that binds to a peptide comprising an amino acid sequence of SEQ ID NO: 3.

24. A method of detecting or quantitating the amount of FGF-like polypeptide using the anti-FGF-like antibody of Claim 21.

25. A method of detecting or quantitating the amount of FGF-like polypeptide using the anti-FGF-like antibody or fragment of Claim 21.

26. The method of claim 25, wherein the antibody or fragment thereof is a monoclonal antibody.

27. A selective binding agent or fragment thereof that specifically binds at least one polypeptide wherein said polypeptide comprises the amino acid sequence selected from the group consisting of:

(a) the amino acid sequence as set forth in SEQ ID NO: 2 or SEQ ID NO: 3; and

(b) a fragment of the amino acid sequence set forth in SEQ ID NO: 2; and

(c) a naturally occurring variant of (a) or (b).

28. The selective binding agent of Claim 27 that is an antibody or fragment thereof.

29. The selective binding agent of Claim 27 that is a humanized antibody.

30. The selective binding agent of Claim 27 that is a human antibody or fragment thereof.

31. The selective binding agent of Claim 27 that is a polyclonal antibody or fragment thereof.

32. The selective binding agent Claim 27 that is a monoclonal antibody or fragment thereof.

33. The selective binding agent of Claim 27 that is a chimeric antibody or fragment thereof.

34. The selective binding agent of Claim 27 that is a CDR-grafted antibody or fragment thereof.

35. The selective binding agent of Claim 27 that is an antiidiotypic antibody or fragment thereof.

36. The selective binding agent of Claim 27 that is a variable region fragment.

37. The variable region fragment of Claim 36 that is a Fab or a Fab' fragment.

38. A selective binding agent or fragment thereof comprising at least one complementarity determining region with specificity for a polypeptide having the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 3.

39. The selective binding agent of Claim 27 that is bound to a detectable label.

40. The selective binding agent of Claim 27 that antagonizes FGF-like polypeptide biological activity.

41. A method for treating, preventing, or ameliorating a disease, condition, or disorder comprising administering to a patient an effective amount of a selective binding agent according to Claim 27.

42. A selective binding agent produced by immunizing

an animal with a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2 or SEQ ID NO: 3.

43. A hybridoma that produces a selective binding agent capable of binding a polypeptide according to Claims 1, 2, or 3.

44. A composition comprising the polypeptide of Claims 15, 16, or 17 and a pharmaceutically acceptable formulation agent.

45. The composition of Claim 44 wherein the pharmaceutically acceptable formulation agent is a carrier, adjuvant, solubilizer, stabilizer, or anti-oxidant.

46. The composition of Claim 44 wherein the polypeptide comprises the mature amino acid sequence as set forth in SEQ ID NO: 3.

47. A polypeptide comprising a derivative of the polypeptide of Claims 15, 16, or 17.

48. The polypeptide of Claim 47 that is covalently modified with a water-soluble polymer.

49. The polypeptide of Claim 48 wherein the water-soluble polymer is selected from polyethylene glycol, monomethoxy-polyethylene glycol, dextran, cellulose, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols, and polyvinyl alcohol.

50. A composition comprising a nucleic acid molecule of Claims 1, 2, or 3 and a pharmaceutically acceptable formulation agent.

51. A composition of Claim 50 wherein said nucleic acid molecule is contained in a viral vector.

52. A viral vector comprising a nucleic acid molecule of Claims 1, 2, or 3.

53. A fusion polypeptide comprising the polypeptide of Claims 15, 16, or 17 fused to a heterologous amino acid sequence.

54. The fusion polypeptide of Claim 53 wherein the heterologous amino acid sequence is an IgG constant domain or fragment thereof.

55. A method for treating, preventing or ameliorating a medical condition comprising administering to a patient the polypeptide of Claims 15, 16, or 17 or the polypeptide encoded by the nucleic acid of Claims 1, 2, or 3.

56. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of Claims 15, 16, or 17 or the polypeptide encoded by the nucleic acid molecule of Claims 1, 2, or 3 in a sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

57. A device, comprising:

(a) a membrane suitable for implantation; and

(b) cells encapsulated within said membrane, wherein said cells secrete a protein of Claims 15, 16, or 17, and wherein said membrane is permeable to said protein and impermeable to materials detrimental to said cells.

58. A method of identifying a compound which binds to a polypeptide comprising:

(a) contacting the polypeptide of Claims 15, 16, or 17 with a compound; and

(b) determining the extent of binding of the polypeptide to the compound.

59. A method of modulating levels of a polypeptide in an animal comprising administering to the animal the nucleic acid molecule of Claims 1, 2, or 3.

60. A transgenic non-human mammal comprising the nucleic acid molecule of Claims 1, 2, or 3.

Figure 1

Human FGF-like Polypeptide Nucleotide and Amino Acid Sequences

```
1    tgccgggtca tgcgccgccg cctgtggctg ggcctggcct ggctgctgct
51   ggcgcgggcg ccggacgccg cgggaacccc gagcgcgtcg cggggaccgc
101  gcagctaccc gcacctggag ggcgacgtgc gctggcggcg cctcttctcc
151  tccactcact tcttcctgcg cgtggatccc ggcggccgcg tgcagggcac
201  ccgctggcgc cacggccagg acagcatcct ggagatccgc tctgtacacg
251  tgggcgtcgt ggtcatcaaa gcagtgtcct caggcttcta cgtggccatg
301  aaccgccggg gccgcctcta cgggtcgcga ctctacaccg tggactgcag
351  gttccgggag cgcatcgaag agaacggcca caacacctac gcctcacagc
401  gctggcgccg ccgcggccag cccatgttcc tggcgctgga caggaggggg
451  gggccccggc caggcggccg gacgcggcgg taccacctgt ccgcccactt
501  cctgcccgtc ctggtctcct gaggccctga gaggccggcg gctccccaag
551  gtgc   (SEQ ID NO: 1)
```

Human FGF-like Polypeptide Precursor:

MRRRLWLGLA WLLLARAPDA AGTPSASRGP RSYPHLEGDV RWRRLFSSTH
FFLRVDPGGR VQGTRWRHGQ DSILEIRSVH VGVVVIKAVS SGFYVAMNRR
GRLYGSRLYT VDCRFRERIE ENGHNTYASQ RWRRRGQPMF LALDRRGGPR
PGGRTRRYHL SAHFLPVLVS (SEQ ID NO: 2)

Mature Human FGF-like Polypeptide:

TPSASRGP RSYPHLEGDV RWRRLFSSTH FFLRVDPGGR VQGTRWRHGQ
DSILEIRSVH VGVVVIKAVS SGFYVAMNRR GRLYGSRLYT VDCRFRERIE
ENGHNTYASQ RWRRRGQPMF LALDRRGGPR PGGRTRRYHL SAHFLPVLVS
(SEQ ID NO: 3)

Figure 2

Murine FGF-like Nucleotide and Amino Acid Sequences

atgcgcagccgcctctggctgggcctagcctggctgctgttggcgcgggcaccgggcgctcc
gggagggtacccgcatctggagggcgacgtgcgctggcgccgcctcttctcctccactcact
ttttcctgcgtgtggaccttggtggtcgggtgcaggggacgcgttggcggcacggccaggac
agtatagtggagatccgttctgtccgtgtgggcactgtggtgatcaaagctgtgtactcagg
cttctatgtggccatgaatcgcaggggccgcctctatgggtcgcgggtctactctgtggact
gtaggttccgggagcgcatcgaggagaacggctacaacacatacgcctcgcgacgttggagg
caccgcggccgacccatgttcctggcacttgacagccaaggcattcccaggcaaggcagacg
gacacgacggcaccaactgtccacacacttcctgccagtcttggtctcgtcttga
(SEQ ID NO: 31)

Murine FGF-like Polypeptide Precursor:

MRSRLWLGLAWLLLARAPGAPGGYPHLEGDVRWRRLFSSTHFFLRVDLGGRVQGTRWRHGQD
SIVEIRSVRVGTVVIKAVYSGFYVAMHRRGRLYGSRVYSVDCRFRERIEENGYNTYASRRWR
HRGRPMFLALDSQGIPRQGRRTRRHQLSTHFLPVLVSS (SEQ ID NO: 32)

Mature Murine FGF-like Polypeptide:

GYPHLEGDVRWRRLFSSTHFFLRVDLGGRVQGTRWRHGQDSIVEIRSVRVGTVVIKAVYSGF
YVAMHRRGRLYGSRVYSVDCRFRERIEENGYNTYASRRWRHRGRPMFLALDSQGIPRQGRRT
RRHQLSTHFLPVLVSS (SEQ ID NO: 33)

Figure 3

Amino Acid Sequences of Some Members of the FGF Family

Human FGF-1:

MAEGEITTFALTEKFNLPPGNYKKPKLLYCSNGGHFLRILPDGTVDGTRDRSDQHIQLQLSA
ESVGEVYIKSTETGQYLAMDTDGLLYGSQTPNEECLFLERLEENHYNTYISKKHAENWFVGL
KKNGSCKRGPRTHYGQKAILFLPLPVSSD (SEQ ID NO: 4)

Human FGF-2:

MAAGSITTLPALPEDGGSGAFPPGHFKDPKRLYCKNGGFFLRIHPDGRVDGVREKSDPHIKL
QLQAEERGVVSIKGVCANRYLAMKEDGRLLASKCVTDECFFFERLESNNYNTYRSRKYTSWY
VALKRTGQYKLGSKTGPGQKAILFLPMSAKS (SEQ ID NO: 5)

Human FGF-3:

MGLIWLLLLSLLEPGWPAAGPGARLRRDAGGRGGVYEHLGGAPRRRKLYCATKYHLQLHPSG
RVNGSLENSAYSILEITAVEVGIVAIRGLFSGRYLAMNKRGRLYASEHYSAECEFVERIHEL
GYNTYASRLYRTVSSTPGARRQPSAERLWYVSVNGKGRPRRGFKTRRTQKSSLFLPRVLDHR
DHEMVRQLQSGLPRPPGKGVQPRRRRQKQSPDNLEPSHVQASRLGSQLEASAH
(SEQ ID NO: 6)

Human FGF-4:

MSGPGTAAVALLPAVLLALLAPWAGRGGAAAPTAPNGTLEAELERRWESLVALSLARLPVAA
QPKEAAVQSGAGDYLLGIKRLRRLYCNVGIGFHLQALPDGRIGGAHADTRDSLLELSPVERG
VVSIFGVASRFFVAMSSKGKLYGSPFFTDECTFKEILLPNNYNAYESYKYPGMFIALSKNGK
TKKGNRVSPTMKVTHFLPRL (SEQ ID NO: 7)

Human FGF-5:

MSLSFLLLLFFSHLILSAWAHGEKRLAPKGQPGPAATDRNPRGSSSRQSSSSAMSSSSASSS
PAASLGSQGSGLEQSSFQWSLGARTGSLYCRVGIGFHLQIYPDGKVNGSHEANMLSVLEIFA
VSQGIVGIRGVFSNKFLAMSKKGKLHASAKFTDDCKFRERFQENSYNTYASAIHRTEKTGRE
WYVALNKRGKAKRGCSPRVKPQHISTHFLPRFKQSEQPELSFTVTVPEKKNPPSPIKSKIPL
SAPRKNTNSVKYRLKFRFG (SEQ ID NO: 8)

Human FGF-6:

MALGQKLFITMSRGAGRLQGTLWALVFLGILVGMVVPSPAGTRANNTLLDSRGWGTLLSRSR
AGLAGEIAGVNWESGYLVGIKRQRRLYCNVGIGFHLQVLPDGRISGTHEENPYSLLEISTVE
RGVVSLFGVRSALFVAMNSKGRLYATPSFQEECKFRETLLPNNYNAYESDLYQGTYIALSKY
GRVKRGSKVSPIMTVTHFLPRI (SEQ ID NO: 9)

Figure 3 (cont.)

Human FGF-7:

MHKWILTWILPTLLYRSCFHIICLVGTISLACNDMTPEQMATNVNCSSPERHTRSYDYMEGG
DIRVRRLFCRTQWYLRIDKRGKVKGTQEMKNNYNIMEIRTVAVGIVAIKGVESEFYLAMNKE
GKLYAKKECNEDCNFKELILENHYNTYASAKWTHNGGEMFVALNQKGIPVRGKKTKKEQKTA
HFLPMAIT (SEQ ID NO: 10)

Human FGF-8:

MGSPRSALSCLLLHLLVLCLQAQEGPGRGPALGRELASLFRAGREPQGVSQQHVREQSLVTD
QLSRRLIRTYQLYSRTSGKHVQVLANKRINAMAEDGDPFAKLIVETDTFGSRVRVRGAETGL
YICMNKKGKLIAKSNGKGKDCVFTEIVLENNYTALQNAKYEGWYMAFTRKGRPRKGSKTRQH
QREVHFMKRLPRGHHTTEQSLRFEFLNYPPFTRSLRGSQRTWAPEPR
(SEQ ID NO: 11)

Human FGF-9:

MAPLGEVGNYFGVQDAVPFGNVPVLPVDSPVLLSDHLGQSEAGGLPRGPAVTDLDH
LKGILRRRQLYCRTGFHLEIFPNGTIQGTRKDHSRFGILEFISIAVGLVSIRGVDSGLYL
GMNEKGELYGSEKLTQECVFREQFEENWYNTYSSNLYKHVDTGRRYYVALNKDGT
PREGTRTKRHQKFTHFLPRPVDPDKVPELYKDILSQS (SEQ ID NO: 12)

Human FGF-10:

MWKWILTHCASAFPHLPGCCCCCFLLLFLVSSVPVTCQALGQDMVSPEATNSSSSSFSSPSS
AGRHVRSYNHLQGDVRWRKLFSFTKYFLKIEKNGKVSGTKKENCPYSILEITSVEIGVVAVK
AINSNYYLAMNKKGKLYGSKEFNNDCKLKERIEENGYNTYASFNWQHNGRQMYVALNGKGAP
RRGQKTRRKNTSAHFLPMVVHS (SEQ ID NO: 13)

Human FGF-11:

MAALASSLIRQKREVREPGGSRPVSAQRRVCPRGTKSLCQKQLLILLSKVRLCGGRPARPDR
GPEPQLKGIVTKLFCRQGFYLQANPDGSIQGTPEDTSSFTHFNLIPVGLRVVTIQSAKLGHY
MAMNAEGLLYSSPHFTAECRFKECVFENYYVLYASALYRQRRSGRAWYLGLDKEGQVMKGNR
VKKTKAAAHFLPKLLEVAMYQEPSLHSVPEASPSSPPAP (SEQ ID NO: 14)

Human FGF-12:

MAAAIASSLIRQKRQARESNSDRVSASKRRSSPSKDGRSLCERHVLGVFSKVRFCSGRKRPV
RRRPEPQLKGIVTRLFSQQGYFLQMHPDGTIDGTKDENSDYTLFNLIPVGLRVVAIQGVKAS
LYVAMNGEGYLYSSDVFTPECKFKESVFENYYVIYSSTLYRQQESGRAWFLGLNKEGQIMKG
NRVKKTKPSSHFVPKPIEVCMYREPSLHEIGEKQGRSRKSSGTPTMNGGKVVNQDST
(SEQ ID NO: 15)

Figure 3 (cont.)

Human FGF-13:

MAAAIASSLIRQKRQARAREKSNACKCVSSPSKGKTSCDKNKLNVFSRVKLFGSKKRRRRRP
EPQLKGIVTKLYSRQGYHLQLQADGTIDGTKDEDSTYTLFNLIPVGLRVVAIQGVQTKLYLA
MNSEGYLYTSELFTPECKFKESVFENYYVTYSSMIYRQQQSGRGWYLGLNKEGEIMKGNHVK
KNKPAAHFLPKPLKVAMYKEPSLHDLTEFSRSGSGTPTKSRSVSGVLNGGKSMSHNEST
(SEQ ID NO: 16)

Human FGF-14:

MAAAIASGLIRQKRQAREQHWDRPSASRRRSSPSKNRGLCNGNLVDIFSKVRIFGLKKRRLR
RQDPQLKGIVTRLYCRQGYYLQMHPDGALDGTKDDSTNSTLFNLIPVGLRVVAIQGVKTGLY
IAMNGEGYLYPSELFTPECKFKESVFENYYVIYSSMLYRQQESGRAWFLGLNKEGQAMKGNR
VKKTKPAAHFLPKPLEVAMYREPSLHDVGETVPKPGVTPSKSTSASAIMNGGKPVNKSKTT
(SEQ ID NO: 17)

Murine FGF-15:

MARKWNGRAVARALVLATLWLAVSGRPLAQQSQSVSDEDPLFLYGWGKITRLQYLYSAGPYV
SNCFLRIRSDGSVDCEEDQNERNLLEFRAVALKTIAIKDVSSVRYLCMSADGKIYGLIRYSE
EDCTFREEMDCLGYNQYRSMKHHLHIIFIQAKPREQLQDQKPSNFIPVFHRSFFETGDQLRS
KMFSLPLESDSMDPFRMVEDVDHLVKSPSFQK (SEQ ID NO: 18)

Human FGF-16:

MAEVGGVFASLDWDLHGFSSSLGNVPLADSPGFLNERLGQIEGKLQRGSPTDFAHLKGILRR
RQLYCRTGFHLEIFPNGTVHGTRHDHSRFGILEFISLAVGLISIRGVDSGLYLGMNERGELY
GSKKLTRECVFREQFEENWYNTYASTLYKHSDSERQYYVALNKDGSPREGYRTKRHQKFTHF
LPRPVDPSKLPSMSRDLFHYR (SEQ ID NO: 19)

Human FGF-17:

TSPAMGAARLLPNLTLCLQLLILCCQTQGENHPSPNFNQYVRDQGAMTDQLSRRQIREYQLY
SRTSGKHVQVTGRRISATAEDGNKFAKLIVETDTFGSRVRIKGAESEKYICMNKRGKLIGKP
SGKSKDCVFTEIVLENNYTAFQNARHEGWFMAFTRQGRPRQASRSRQNQREAHFIKRLYQGQ
LPFPNHAEKQKQFEFVGSAPTRRTKRTRRPQPLT (SEQ ID NO: 20)

Human FGF-18:

MYSAPSACTCLCLHFLLLCFQVQVLVAEENVDFRIHVENQTRARDDVSRKQLRLYQLYSRTS
GKHIQVLGRRISARGEDGDKYAQLLVETDTFGSQVRIKGKETEFYLCMNRKGKLVGKPDGTS
KECVFIEKVLENNYTALMSAKYSGWYVGFTKKGRPRKGPKTRENQQDVHFMKRYPKGQPELQ
KPFKYTTVTKRSRRIRPTHPA (SEQ ID NO: 21)

Human FGF-19:

MRSGCVVVHVWILAGLWLAVAGRPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRI
RADGVVDCARGQSAHSLLEIKAVALRTVAIKGVHSVRYLCMGADGKMQGLLQYSEEDCAFEE
EIRPDGYNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESD
MFSSPLETDSMDPFGLVTGLEAVRSPSFEK (SEQ ID NO: 22)

Figure 3 (cont.)

Human FGF-20:

MAPLAEVGGFLGGLEGLGQQVGSHFLLPPAGERPPLLGERRSAAERSARGGPGAAQLAHLHG
ILRRRQLYCRTGFHLQILPDGSVQGTRQDHSLFGILEFISVAVGLVSIRGVDSGLYLGMNDK
GELYGSEKLTSECIFREQFEENWYNTYSSNIYKHGDTGRRYFVALNKDGTPRDGARSKRHQK
FTHFLPRPVDPERVPELYKDLLMYT (SEQ ID NO: 23)

Human FGF-21:

MDSDETGFEHSGLWVSVLAGLLLGACQAHPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLE
IREDGTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFR
ELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPP
DVGSSDPLSMVGPSQGRSPSYAS (SEQ ID NO: 24)

SEQUENCE LISTING

<110> Itoh, Nobuyuki

<120> Novel Fibroblast Growth Factor-Like Polypeptides

<130> 08035.0001-01304

<140>
<141>

<160> 35

<170> PatentIn Ver. 2.0

<210> 1
<211> 554
<212> DNA
<213> Homo sapiens

<400> 1
tgccgggtca tgcgccgccg cctgtggctg ggcctggcct ggctgctgct ggcgcgggcg 60
ccggacgccg cgggaacccc gagcgcgtcg cggggaccgc gcagctaccc gcacctggag 120
ggcgacgtgc gctggcggcg cctcttctcc tccactcact tcttcctgcg cgtggatccc 180
ggcggccgcg tgcagggcac ccgctggcgc cacggccagg acagcatcct ggagatccgc 240
tctgtacacg tgggcgtcgt ggtcatcaaa gcagtgtcct caggcttcta cgtggccatg 300
aaccgccggg gccgcctcta cgggtcgcga ctctacaccg tggactgcag gttccgggag 360
cgcatcgaag agaacggcca caacacctac gcctcacagc gctggcgccg ccgcggccag 420
cccatgttcc tggcgctgga caggaggggg gggccccggc caggcggccg gacgcggcgg 480
taccacctgt ccgcccactt cctgcccgtc ctggtctcct gaggccctga gaggccggcg 540
gctccccaag gtgc 554

<210> 2
<211> 170
<212> PRT
<213> Homo sapiens

<400> 2
Met Arg Arg Arg Leu Trp Leu Gly Leu Ala Trp Leu Leu Leu Ala Arg
1 5 10 15

Ala Pro Asp Ala Ala Gly Thr Pro Ser Ala Ser Arg Gly Pro Arg Ser
20 25 30

Tyr Pro His Leu Glu Gly Asp Val Arg Trp Arg Arg Leu Phe Ser Ser
35 40 45

Thr His Phe Phe Leu Arg Val Asp Pro Gly Gly Arg Val Gln Gly Thr
50 55 60

Arg Trp Arg His Gly Gln Asp Ser Ile Leu Glu Ile Arg Ser Val His
65 70 75 80

Val Gly Val Val Val Ile Lys Ala Val Ser Ser Gly Phe Tyr Val Ala
85 90 95

Met Asn Arg Arg Gly Arg Leu Tyr Gly Ser Arg Leu Tyr Thr Val Asp
100 105 110

```
Cys Arg Phe Arg Glu Arg Ile Glu Glu Asn Gly His Asn Thr Tyr Ala
        115                 120                 125

Ser Gln Arg Trp Arg Arg Arg Gly Gln Pro Met Phe Leu Ala Leu Asp
    130                 135                 140

Arg Arg Gly Gly Pro Arg Pro Gly Gly Arg Thr Arg Arg Tyr His Leu
145                 150                 155                 160

Ser Ala His Phe Leu Pro Val Leu Val Ser
                165                 170
```

<210> 3
<211> 148
<212> PRT
<213> Homo sapiens

<400> 3

```
Thr Pro Ser Ala Ser Arg Gly Pro Arg Ser Tyr Pro His Leu Glu Gly
  1               5                  10                  15

Asp Val Arg Trp Arg Arg Leu Phe Ser Ser Thr His Phe Phe Leu Arg
             20                  25                  30

Val Asp Pro Gly Gly Arg Val Gln Gly Thr Arg Trp Arg His Gly Gln
         35                  40                  45

Asp Ser Ile Leu Glu Ile Arg Ser Val His Val Gly Val Val Val Ile
     50                  55                  60

Lys Ala Val Ser Ser Gly Phe Tyr Val Ala Met Asn Arg Arg Gly Arg
 65                  70                  75                  80

Leu Tyr Gly Ser Arg Leu Tyr Thr Val Asp Cys Arg Phe Arg Glu Arg
                85                  90                  95

Ile Glu Glu Asn Gly His Asn Thr Tyr Ala Ser Gln Arg Trp Arg Arg
            100                 105                 110

Arg Gly Gln Pro Met Phe Leu Ala Leu Asp Arg Arg Gly Gly Pro Arg
        115                 120                 125

Pro Gly Gly Arg Thr Arg Arg Tyr His Leu Ser Ala His Phe Leu Pro
    130                 135                 140

Val Leu Val Ser
145
```

<210> 4
<211> 153
<212> PRT
<213> Homo sapiens

<300>
<303> Biochem. Biophys. Res. Commun.
<304> 138
<306> 611-617
<307> 1986

<400> 4
Met Ala Glu Gly Glu Ile Thr Thr Phe Ala Leu Thr Glu Lys Phe Asn
1 5 10 15

Leu Pro Pro Gly Asn Tyr Lys Lys Pro Lys Leu Leu Tyr Cys Ser Asn
20 25 30

Gly Gly His Phe Leu Arg Ile Leu Pro Asp Gly Thr Val Asp Gly Thr
35 40 45

Arg Asp Arg Ser Asp Gln His Ile Gln Leu Gln Leu Ser Ala Glu Ser
50 55 60

Val Gly Glu Val Tyr Ile Lys Ser Thr Glu Thr Gly Gln Tyr Leu Ala
65 70 75 80

Met Asp Thr Asp Gly Leu Leu Tyr Gly Ser Gln Thr Pro Asn Glu Glu
85 90 95

Cys Leu Phe Leu Glu Arg Leu Glu Glu Asn His Tyr Asn Thr Tyr Ile
100 105 110

Ser Lys Lys His Ala Glu Asn Trp Phe Val Gly Leu Lys Lys Asn Gly
115 120 125

Ser Cys Lys Arg Gly Pro Arg Thr His Tyr Gly Gln Lys Ala Ile Leu
130 135 140

Phe Leu Pro Leu Pro Val Ser Ser Asp
145 150

<210> 5
<211> 155
<212> PRT
<213> Homo sapiens

<300>
<303> EMBO J.
<304> 5
<306> 2523-2528
<307> 1986

<400> 5
Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly
1 5 10 15

Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu
20 25 30

Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg
35 40 45

Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu
50 55 60

Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn
65 70 75 80

Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys

85 90 95

Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr
100 105 110

Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys
115 120 125

Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys
130 135 140

Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser
145 150 155

<210> 6
<211> 239
<212> PRT
<213> Homo sapiens

<300>
<303> Ann. N. Y. Acad. Sci.
<304> 638
<306> 18-26
<307> 1991

<400> 6
Met Gly Leu Ile Trp Leu Leu Leu Leu Ser Leu Leu Glu Pro Gly Trp
1 5 10 15

Pro Ala Ala Gly Pro Gly Ala Arg Leu Arg Arg Asp Ala Gly Gly Arg
20 25 30

Gly Gly Val Tyr Glu His Leu Gly Gly Ala Pro Arg Arg Arg Lys Leu
35 40 45

Tyr Cys Ala Thr Lys Tyr His Leu Gln Leu His Pro Ser Gly Arg Val
50 55 60

Asn Gly Ser Leu Glu Asn Ser Ala Tyr Ser Ile Leu Glu Ile Thr Ala
65 70 75 80

Val Glu Val Gly Ile Val Ala Ile Arg Gly Leu Phe Ser Gly Arg Tyr
85 90 95

Leu Ala Met Asn Lys Arg Gly Arg Leu Tyr Ala Ser Glu His Tyr Ser
100 105 110

Ala Glu Cys Glu Phe Val Glu Arg Ile His Glu Leu Gly Tyr Asn Thr
115 120 125

Tyr Ala Ser Arg Leu Tyr Arg Thr Val Ser Ser Thr Pro Gly Ala Arg
130 135 140

Arg Gln Pro Ser Ala Glu Arg Leu Trp Tyr Val Ser Val Asn Gly Lys
145 150 155 160

Gly Arg Pro Arg Arg Gly Phe Lys Thr Arg Arg Thr Gln Lys Ser Ser
165 170 175

Leu Phe Leu Pro Arg Val Leu Asp His Arg Asp His Glu Met Val Arg
180 185 190

Gln Leu Gln Ser Gly Leu Pro Arg Pro Pro Gly Lys Gly Val Gln Pro
195 200 205

Arg Arg Arg Arg Gln Lys Gln Ser Pro Asp Asn Leu Glu Pro Ser His
210 215 220

Val Gln Ala Ser Arg Leu Gly Ser Gln Leu Glu Ala Ser Ala His
225 230 235

<210> 7
<211> 206
<212> PRT
<213> Homo sapiens

<300>
<303> Ann. N. Y. Acad. Sci.
<304> 638
<306> 27-37
<307> 1991

<400> 7
Met Ser Gly Pro Gly Thr Ala Ala Val Ala Leu Leu Pro Ala Val Leu
1 5 10 15

Leu Ala Leu Leu Ala Pro Trp Ala Gly Arg Gly Gly Ala Ala Ala Pro
20 25 30

Thr Ala Pro Asn Gly Thr Leu Glu Ala Glu Leu Glu Arg Arg Trp Glu
35 40 45

Ser Leu Val Ala Leu Ser Leu Ala Arg Leu Pro Val Ala Ala Gln Pro
50 55 60

Lys Glu Ala Ala Val Gln Ser Gly Ala Gly Asp Tyr Leu Leu Gly Ile
65 70 75 80

Lys Arg Leu Arg Arg Leu Tyr Cys Asn Val Gly Ile Gly Phe His Leu
85 90 95

Gln Ala Leu Pro Asp Gly Arg Ile Gly Gly Ala His Ala Asp Thr Arg
100 105 110

Asp Ser Leu Leu Glu Leu Ser Pro Val Glu Arg Gly Val Val Ser Ile
115 120 125

Phe Gly Val Ala Ser Arg Phe Phe Val Ala Met Ser Ser Lys Gly Lys
130 135 140

Leu Tyr Gly Ser Pro Phe Phe Thr Asp Glu Cys Thr Phe Lys Glu Ile
145 150 155 160

Leu Leu Pro Asn Asn Tyr Asn Ala Tyr Glu Ser Tyr Lys Tyr Pro Gly
165 170 175

Met Phe Ile Ala Leu Ser Lys Asn Gly Lys Thr Lys Lys Gly Asn Arg
180 185 190

Val Ser Pro Thr Met Lys Val Thr His Phe Leu Pro Arg Leu
195 200 205

<210> 8
<211> 267
<212> PRT
<213> Homo sapiens

<300>
<303> Ann. N. Y. Acad. Sci.
<304> 638
<306> 38-52
<307> 1991

<400> 8
Met Ser Leu Ser Phe Leu Leu Leu Leu Phe Phe Ser His Leu Ile Leu
1 5 10 15

Ser Ala Trp Ala His Gly Glu Lys Arg Leu Ala Pro Lys Gly Gln Pro
20 25 30

Gly Pro Ala Ala Thr Asp Arg Asn Pro Arg Gly Ser Ser Ser Arg Gln
35 40 45

Ser Ser Ser Ser Ala Met Ser Ser Ser Ser Ala Ser Ser Ser Pro Ala
50 55 60

Ala Ser Leu Gly Ser Gln Gly Ser Gly Leu Glu Gln Ser Ser Phe Gln
65 70 75 80

Trp Ser Leu Gly Ala Arg Thr Gly Ser Leu Tyr Cys Arg Val Gly Ile
85 90 95

Gly Phe His Leu Gln Ile Tyr Pro Asp Gly Lys Val Asn Gly Ser His
100 105 110

Glu Ala Asn Met Leu Ser Val Leu Glu Ile Phe Ala Val Ser Gln Gly
115 120 125

Ile Val Gly Ile Arg Gly Val Phe Ser Asn Lys Phe Leu Ala Met Ser
130 135 140

Lys Lys Gly Lys Leu His Ala Ser Ala Lys Phe Thr Asp Asp Cys Lys
145 150 155 160

Phe Arg Glu Arg Phe Gln Glu Asn Ser Tyr Asn Thr Tyr Ala Ser Ala
165 170 175

Ile His Arg Thr Glu Lys Thr Gly Arg Glu Trp Tyr Val Ala Leu Asn
180 185 190

Lys Arg Gly Lys Ala Lys Arg Gly Cys Ser Pro Arg Val Lys Pro Gln
195 200 205

His Ile Ser Thr His Phe Leu Pro Arg Phe Lys Gln Ser Glu Gln Pro
210 215 220

Glu Leu Ser Phe Thr Val Thr Val Pro Glu Lys Lys Asn Pro Pro Ser

225 230 235 240

Pro Ile Lys Ser Lys Ile Pro Leu Ser Ala Pro Arg Lys Asn Thr Asn
245 250 255

Ser Val Lys Tyr Arg Leu Lys Phe Arg Phe Gly
260 265

<210> 9
<211> 208
<212> PRT
<213> Homo sapiens

<300>
<303> Ann. N. Y. Acad. Sci.
<304> 638
<306> 53-61
<307> 1991

<400> 9
Met Ala Leu Gly Gln Lys Leu Phe Ile Thr Met Ser Arg Gly Ala Gly
1 5 10 15

Arg Leu Gln Gly Thr Leu Trp Ala Leu Val Phe Leu Gly Ile Leu Val
20 25 30

Gly Met Val Val Pro Ser Pro Ala Gly Thr Arg Ala Asn Asn Thr Leu
35 40 45

Leu Asp Ser Arg Gly Trp Gly Thr Leu Leu Ser Arg Ser Arg Ala Gly
50 55 60

Leu Ala Gly Glu Ile Ala Gly Val Asn Trp Glu Ser Gly Tyr Leu Val
65 70 75 80

Gly Ile Lys Arg Gln Arg Arg Leu Tyr Cys Asn Val Gly Ile Gly Phe
85 90 95

His Leu Gln Val Leu Pro Asp Gly Arg Ile Ser Gly Thr His Glu Glu
100 105 110

Asn Pro Tyr Ser Leu Leu Glu Ile Ser Thr Val Glu Arg Gly Val Val
115 120 125

Ser Leu Phe Gly Val Arg Ser Ala Leu Phe Val Ala Met Asn Ser Lys
130 135 140

Gly Arg Leu Tyr Ala Thr Pro Ser Phe Gln Glu Glu Cys Lys Phe Arg
145 150 155 160

Glu Thr Leu Leu Pro Asn Asn Tyr Asn Ala Tyr Glu Ser Asp Leu Tyr
165 170 175

Gln Gly Thr Tyr Ile Ala Leu Ser Lys Tyr Gly Arg Val Lys Arg Gly
180 185 190

Ser Lys Val Ser Pro Ile Met Thr Val Thr His Phe Leu Pro Arg Ile
195 200 205

<210> 10
<211> 194
<212> PRT
<213> Homo sapiens

<300>
<303> Ann. N. Y. Acad. Sci.
<304> 638
<306> 62-77
<307> 1991

<400> 10
Met His Lys Trp Ile Leu Thr Trp Ile Leu Pro Thr Leu Leu Tyr Arg
1 5 10 15

Ser Cys Phe His Ile Ile Cys Leu Val Gly Thr Ile Ser Leu Ala Cys
20 25 30

Asn Asp Met Thr Pro Glu Gln Met Ala Thr Asn Val Asn Cys Ser Ser
35 40 45

Pro Glu Arg His Thr Arg Ser Tyr Asp Tyr Met Glu Gly Gly Asp Ile
50 55 60

Arg Val Arg Arg Leu Phe Cys Arg Thr Gln Trp Tyr Leu Arg Ile Asp
65 70 75 80

Lys Arg Gly Lys Val Lys Gly Thr Gln Glu Met Lys Asn Asn Tyr Asn
85 90 95

Ile Met Glu Ile Arg Thr Val Ala Val Gly Ile Val Ala Ile Lys Gly
100 105 110

Val Glu Ser Glu Phe Tyr Leu Ala Met Asn Lys Glu Gly Lys Leu Tyr
115 120 125

Ala Lys Lys Glu Cys Asn Glu Asp Cys Asn Phe Lys Glu Leu Ile Leu
130 135 140

Glu Asn His Tyr Asn Thr Tyr Ala Ser Ala Lys Trp Thr His Asn Gly
145 150 155 160

Gly Glu Met Phe Val Ala Leu Asn Gln Lys Gly Ile Pro Val Arg Gly
165 170 175

Lys Lys Thr Lys Lys Glu Gln Lys Thr Ala His Phe Leu Pro Met Ala
180 185 190

Ile Thr

<210> 11
<211> 233
<212> PRT
<213> Homo sapiens

<300>
<303> Proc. Natl. Acad. Sci. U.S.A.
<304> 89

<306> 8928-8932
<307> 1992

<400> 11
Met Gly Ser Pro Arg Ser Ala Leu Ser Cys Leu Leu Leu His Leu Leu
1 5 10 15

Val Leu Cys Leu Gln Ala Gln Glu Gly Pro Gly Arg Gly Pro Ala Leu
20 25 30

Gly Arg Glu Leu Ala Ser Leu Phe Arg Ala Gly Arg Glu Pro Gln Gly
35 40 45

Val Ser Gln Gln His Val Arg Glu Gln Ser Leu Val Thr Asp Gln Leu
50 55 60

Ser Arg Arg Leu Ile Arg Thr Tyr Gln Leu Tyr Ser Arg Thr Ser Gly
65 70 75 80

Lys His Val Gln Val Leu Ala Asn Lys Arg Ile Asn Ala Met Ala Glu
85 90 95

Asp Gly Asp Pro Phe Ala Lys Leu Ile Val Glu Thr Asp Thr Phe Gly
100 105 110

Ser Arg Val Arg Val Arg Gly Ala Glu Thr Gly Leu Tyr Ile Cys Met
115 120 125

Asn Lys Lys Gly Lys Leu Ile Ala Lys Ser Asn Gly Lys Gly Lys Asp
130 135 140

Cys Val Phe Thr Glu Ile Val Leu Glu Asn Asn Tyr Thr Ala Leu Gln
145 150 155 160

Asn Ala Lys Tyr Glu Gly Trp Tyr Met Ala Phe Thr Arg Lys Gly Arg
165 170 175

Pro Arg Lys Gly Ser Lys Thr Arg Gln His Gln Arg Glu Val His Phe
180 185 190

Met Lys Arg Leu Pro Arg Gly His His Thr Thr Glu Gln Ser Leu Arg
195 200 205

Phe Glu Phe Leu Asn Tyr Pro Pro Phe Thr Arg Ser Leu Arg Gly Ser
210 215 220

Gln Arg Thr Trp Ala Pro Glu Pro Arg
225 230

<210> 12
<211> 208
<212> PRT
<213> Homo sapiens

<300>
<303> Mol. Cell. Biol.
<304> 4251
<305> 4259
<307> 1993

<400> 12
Met Ala Pro Leu Gly Glu Val Gly Asn Tyr Phe Gly Val Gln Asp Ala
1 5 10 15

Val Pro Phe Gly Asn Val Pro Val Leu Pro Val Asp Ser Pro Val Leu
20 25 30

Leu Ser Asp His Leu Gly Gln Ser Glu Ala Gly Gly Leu Pro Arg Gly
35 40 45

Pro Ala Val Thr Asp Leu Asp His Leu Lys Gly Ile Leu Arg Arg Arg
50 55 60

Gln Leu Tyr Cys Arg Thr Gly Phe His Leu Glu Ile Phe Pro Asn Gly
65 70 75 80

Thr Ile Gln Gly Thr Arg Lys Asp His Ser Arg Phe Gly Ile Leu Glu
85 90 95

Phe Ile Ser Ile Ala Val Gly Leu Val Ser Ile Arg Gly Val Asp Ser
100 105 110

Gly Leu Tyr Leu Gly Met Asn Glu Lys Gly Glu Leu Tyr Gly Ser Glu
115 120 125

Lys Leu Thr Gln Glu Cys Val Phe Arg Glu Gln Phe Glu Glu Asn Trp
130 135 140

Tyr Asn Thr Tyr Ser Ser Asn Leu Tyr Lys His Val Asp Thr Gly Arg
145 150 155 160

Arg Tyr Tyr Val Ala Leu Asn Lys Asp Gly Thr Pro Arg Glu Gly Thr
165 170 175

Arg Thr Lys Arg His Gln Lys Phe Thr His Phe Leu Pro Arg Pro Val
180 185 190

Asp Pro Asp Lys Val Pro Glu Leu Tyr Lys Asp Ile Leu Ser Gln Ser
195 200 205

<210> 13
<211> 208
<212> PRT
<213> Homo sapiens

<300>
<303> J. Biol. Chem.
<304> 272
<306> 23191-23194
<307> 1997

<400> 13
Met Trp Lys Trp Ile Leu Thr His Cys Ala Ser Ala Phe Pro His Leu
1 5 10 15

Pro Gly Cys Cys Cys Cys Cys Phe Leu Leu Leu Phe Leu Val Ser Ser
20 25 30

Val Pro Val Thr Cys Gln Ala Leu Gly Gln Asp Met Val Ser Pro Glu
35 40 45

Ala Thr Asn Ser Ser Ser Ser Ser Phe Ser Ser Pro Ser Ser Ala Gly
50 55 60

Arg His Val Arg Ser Tyr Asn His Leu Gln Gly Asp Val Arg Trp Arg
65 70 75 80

Lys Leu Phe Ser Phe Thr Lys Tyr Phe Leu Lys Ile Glu Lys Asn Gly
85 90 95

Lys Val Ser Gly Thr Lys Lys Glu Asn Cys Pro Tyr Ser Ile Leu Glu
100 105 110

Ile Thr Ser Val Glu Ile Gly Val Val Ala Val Lys Ala Ile Asn Ser
115 120 125

Asn Tyr Tyr Leu Ala Met Asn Lys Lys Gly Lys Leu Tyr Gly Ser Lys
130 135 140

Glu Phe Asn Asn Asp Cys Lys Leu Lys Glu Arg Ile Glu Glu Asn Gly
145 150 155 160

Tyr Asn Thr Tyr Ala Ser Phe Asn Trp Gln His Asn Gly Arg Gln Met
165 170 175

Tyr Val Ala Leu Asn Gly Lys Gly Ala Pro Arg Arg Gly Gln Lys Thr
180 185 190

Arg Arg Lys Asn Thr Ser Ala His Phe Leu Pro Met Val Val His Ser
195 200 205

<210> 14
<211> 225
<212> PRT
<213> Homo sapiens

<300>
<303> Proc. Natl. Acad. Sci. U.S.A.
<304> 93
<306> 9850-9857
<307> 1996

<400> 14
Met Ala Ala Leu Ala Ser Ser Leu Ile Arg Gln Lys Arg Glu Val Arg
1 5 10 15

Glu Pro Gly Gly Ser Arg Pro Val Ser Ala Gln Arg Arg Val Cys Pro
20 25 30

Arg Gly Thr Lys Ser Leu Cys Gln Lys Gln Leu Leu Ile Leu Leu Ser
35 40 45

Lys Val Arg Leu Cys Gly Gly Arg Pro Ala Arg Pro Asp Arg Gly Pro
50 55 60

Glu Pro Gln Leu Lys Gly Ile Val Thr Lys Leu Phe Cys Arg Gln Gly
65 70 75 80

```
Phe Tyr Leu Gln Ala Asn Pro Asp Gly Ser Ile Gln Gly Thr Pro Glu
                85                  90                  95

Asp Thr Ser Ser Phe Thr His Phe Asn Leu Ile Pro Val Gly Leu Arg
            100                 105                 110

Val Val Thr Ile Gln Ser Ala Lys Leu Gly His Tyr Met Ala Met Asn
        115                 120                 125

Ala Glu Gly Leu Leu Tyr Ser Ser Pro His Phe Thr Ala Glu Cys Arg
    130                 135                 140

Phe Lys Glu Cys Val Phe Glu Asn Tyr Tyr Val Leu Tyr Ala Ser Ala
145                 150                 155                 160

Leu Tyr Arg Gln Arg Arg Ser Gly Arg Ala Trp Tyr Leu Gly Leu Asp
                165                 170                 175

Lys Glu Gly Gln Val Met Lys Gly Asn Arg Val Lys Lys Thr Lys Ala
            180                 185                 190

Ala Ala His Phe Leu Pro Lys Leu Leu Glu Val Ala Met Tyr Gln Glu
        195                 200                 205

Pro Ser Leu His Ser Val Pro Glu Ala Ser Pro Ser Ser Pro Pro Ala
    210                 215                 220

Pro
225
```

<210> 15
<211> 243
<212> PRT
<213> Homo sapiens

<400> 15

```
Met Ala Ala Ala Ile Ala Ser Ser Leu Ile Arg Gln Lys Arg Gln Ala
  1               5                  10                  15

Arg Glu Ser Asn Ser Asp Arg Val Ser Ala Ser Lys Arg Arg Ser Ser
             20                  25                  30

Pro Ser Lys Asp Gly Arg Ser Leu Cys Glu Arg His Val Leu Gly Val
         35                  40                  45

Phe Ser Lys Val Arg Phe Cys Ser Gly Arg Lys Arg Pro Val Arg Arg
     50                  55                  60

Arg Pro Glu Pro Gln Leu Lys Gly Ile Val Thr Arg Leu Phe Ser Gln
 65                  70                  75                  80

Gln Gly Tyr Phe Leu Gln Met His Pro Asp Gly Thr Ile Asp Gly Thr
                85                  90                  95

Lys Asp Glu Asn Ser Asp Tyr Thr Leu Phe Asn Leu Ile Pro Val Gly
            100                 105                 110

Leu Arg Val Val Ala Ile Gln Gly Val Lys Ala Ser Leu Tyr Val Ala
```

```
        115                 120                 125

Met Asn Gly Glu Gly Tyr Leu Tyr Ser Ser Asp Val Phe Thr Pro Glu
    130                 135                 140

Cys Lys Phe Lys Glu Ser Val Phe Glu Asn Tyr Tyr Val Ile Tyr Ser
145                 150                 155                 160

Ser Thr Leu Tyr Arg Gln Gln Glu Ser Gly Arg Ala Trp Phe Leu Gly
                165                 170                 175

Leu Asn Lys Glu Gly Gln Ile Met Lys Gly Asn Arg Val Lys Lys Thr
            180                 185                 190

Lys Pro Ser Ser His Phe Val Pro Lys Pro Ile Glu Val Cys Met Tyr
        195                 200                 205

Arg Glu Pro Ser Leu His Glu Ile Gly Glu Lys Gln Gly Arg Ser Arg
    210                 215                 220

Lys Ser Ser Gly Thr Pro Thr Met Asn Gly Gly Lys Val Val Asn Gln
225                 230                 235                 240

Asp Ser Thr

<210> 16
<211> 245
<212> PRT
<213> Homo sapiens

<400> 16
Met Ala Ala Ala Ile Ala Ser Ser Leu Ile Arg Gln Lys Arg Gln Ala
  1               5                  10                  15

Arg Glu Arg Glu Lys Ser Asn Ala Cys Lys Cys Val Ser Ser Pro Ser
             20                  25                  30

Lys Gly Lys Thr Ser Cys Asp Lys Asn Lys Leu Asn Val Phe Ser Arg
         35                  40                  45

Val Lys Leu Phe Gly Ser Lys Lys Arg Arg Arg Arg Arg Pro Glu Pro
     50                  55                  60

Gln Leu Lys Gly Ile Val Thr Lys Leu Tyr Ser Arg Gln Gly Tyr His
 65                  70                  75                  80

Leu Gln Leu Gln Ala Asp Gly Thr Ile Asp Gly Thr Lys Asp Glu Asp
                85                  90                  95

Ser Thr Tyr Thr Leu Phe Asn Leu Ile Pro Val Gly Leu Arg Val Val
            100                 105                 110

Ala Ile Gln Gly Val Gln Thr Lys Leu Tyr Leu Ala Met Asn Ser Glu
        115                 120                 125

Gly Tyr Leu Tyr Thr Ser Glu Leu Phe Thr Pro Glu Cys Lys Phe Lys
    130                 135                 140

Glu Ser Val Phe Glu Asn Tyr Tyr Val Thr Tyr Ser Ser Met Ile Tyr
```

145 150 155 160

Arg Gln Gln Gln Ser Gly Arg Gly Trp Tyr Leu Gly Leu Asn Lys Glu
165 170 175

Gly Glu Ile Met Lys Gly Asn His Val Lys Lys Asn Lys Pro Ala Ala
180 185 190

His Phe Leu Pro Lys Pro Leu Lys Val Ala Met Tyr Lys Glu Pro Ser
195 200 205

Leu His Asp Leu Thr Glu Phe Ser Arg Ser Gly Ser Gly Thr Pro Thr
210 215 220

Lys Ser Arg Ser Val Ser Gly Val Leu Asn Gly Gly Lys Ser Met Ser
225 230 235 240

His Asn Glu Ser Thr
245

<210> 17
<211> 247
<212> PRT
<213> Homo sapiens

<400> 17
Met Ala Ala Ala Ile Ala Ser Gly Leu Ile Arg Gln Lys Arg Gln Ala
1 5 10 15

Arg Glu Gln His Trp Asp Arg Pro Ser Ala Ser Arg Arg Arg Ser Ser
20 25 30

Pro Ser Lys Asn Arg Gly Leu Cys Asn Gly Asn Leu Val Asp Ile Phe
35 40 45

Ser Lys Val Arg Ile Phe Gly Leu Lys Lys Arg Arg Leu Arg Arg Gln
50 55 60

Asp Pro Gln Leu Lys Gly Ile Val Thr Arg Leu Tyr Cys Arg Gln Gly
65 70 75 80

Tyr Tyr Leu Gln Met His Pro Asp Gly Ala Leu Asp Gly Thr Lys Asp
85 90 95

Asp Ser Thr Asn Ser Thr Leu Phe Asn Leu Ile Pro Val Gly Leu Arg
100 105 110

Val Val Ala Ile Gln Gly Val Lys Thr Gly Leu Tyr Ile Ala Met Asn
115 120 125

Gly Glu Gly Tyr Leu Tyr Pro Ser Glu Leu Phe Thr Pro Glu Cys Lys
130 135 140

Phe Lys Glu Ser Val Phe Glu Asn Tyr Tyr Val Ile Tyr Ser Ser Met
145 150 155 160

Leu Tyr Arg Gln Gln Glu Ser Gly Arg Ala Trp Phe Leu Gly Leu Asn
165 170 175

```
Lys Glu Gly Gln Ala Met Lys Gly Asn Arg Val Lys Lys Thr Lys Pro
            180                 185                 190

Ala Ala His Phe Leu Pro Lys Pro Leu Glu Val Ala Met Tyr Arg Glu
        195                 200                 205

Pro Ser Leu His Asp Val Gly Glu Thr Val Pro Lys Pro Gly Val Thr
    210                 215                 220

Pro Ser Lys Ser Thr Ser Ala Ser Ala Ile Met Asn Gly Gly Lys Pro
225                 230                 235                 240

Val Asn Lys Ser Lys Thr Thr
                245
```

<210> 18
<211> 218
<212> PRT
<213> Mus sp.

<400> 18

```
Met Ala Arg Lys Trp Asn Gly Arg Ala Val Ala Arg Ala Leu Val Leu
  1               5                  10                  15

Ala Thr Leu Trp Leu Ala Val Ser Gly Arg Pro Leu Ala Gln Gln Ser
            20                  25                  30

Gln Ser Val Ser Asp Glu Asp Pro Leu Phe Leu Tyr Gly Trp Gly Lys
        35                  40                  45

Ile Thr Arg Leu Gln Tyr Leu Tyr Ser Ala Gly Pro Tyr Val Ser Asn
    50                  55                  60

Cys Phe Leu Arg Ile Arg Ser Asp Gly Ser Val Asp Cys Glu Glu Asp
65                  70                  75                  80

Gln Asn Glu Arg Asn Leu Leu Glu Phe Arg Ala Val Ala Leu Lys Thr
                85                  90                  95

Ile Ala Ile Lys Asp Val Ser Ser Val Arg Tyr Leu Cys Met Ser Ala
            100                 105                 110

Asp Gly Lys Ile Tyr Gly Leu Ile Arg Tyr Ser Glu Glu Asp Cys Thr
        115                 120                 125

Phe Arg Glu Glu Met Asp Cys Leu Gly Tyr Asn Gln Tyr Arg Ser Met
    130                 135                 140

Lys His His Leu His Ile Ile Phe Ile Gln Ala Lys Pro Arg Glu Gln
145                 150                 155                 160

Leu Gln Asp Gln Lys Pro Ser Asn Phe Ile Pro Val Phe His Arg Ser
                165                 170                 175

Phe Phe Glu Thr Gly Asp Gln Leu Arg Ser Lys Met Phe Ser Leu Pro
            180                 185                 190

Leu Glu Ser Asp Ser Met Asp Pro Phe Arg Met Val Glu Asp Val Asp
        195                 200                 205
```

His Leu Val Lys Ser Pro Ser Phe Gln Lys
210 215

<210> 19
<211> 207
<212> PRT
<213> Homo sapiens

<400> 19
Met Ala Glu Val Gly Gly Val Phe Ala Ser Leu Asp Trp Asp Leu His
1 5 10 15

Gly Phe Ser Ser Ser Leu Gly Asn Val Pro Leu Ala Asp Ser Pro Gly
20 25 30

Phe Leu Asn Glu Arg Leu Gly Gln Ile Glu Gly Lys Leu Gln Arg Gly
35 40 45

Ser Pro Thr Asp Phe Ala His Leu Lys Gly Ile Leu Arg Arg Arg Gln
50 55 60

Leu Tyr Cys Arg Thr Gly Phe His Leu Glu Ile Phe Pro Asn Gly Thr
65 70 75 80

Val His Gly Thr Arg His Asp His Ser Arg Phe Gly Ile Leu Glu Phe
85 90 95

Ile Ser Leu Ala Val Gly Leu Ile Ser Ile Arg Gly Val Asp Ser Gly
100 105 110

Leu Tyr Leu Gly Met Asn Glu Arg Gly Glu Leu Tyr Gly Ser Lys Lys
115 120 125

Leu Thr Arg Glu Cys Val Phe Arg Glu Gln Phe Glu Glu Asn Trp Tyr
130 135 140

Asn Thr Tyr Ala Ser Thr Leu Tyr Lys His Ser Asp Ser Glu Arg Gln
145 150 155 160

Tyr Tyr Val Ala Leu Asn Lys Asp Gly Ser Pro Arg Glu Gly Tyr Arg
165 170 175

Thr Lys Arg His Gln Lys Phe Thr His Phe Leu Pro Arg Pro Val Asp
180 185 190

Pro Ser Lys Leu Pro Ser Met Ser Arg Asp Leu Phe His Tyr Arg
195 200 205

<210> 20
<211> 220
<212> PRT
<213> Homo sapiens

<400> 20
Thr Ser Pro Ala Met Gly Ala Ala Arg Leu Leu Pro Asn Leu Thr Leu
1 5 10 15

Cys Leu Gln Leu Leu Ile Leu Cys Cys Gln Thr Gln Gly Glu Asn His

```
            20                  25                  30

Pro Ser Pro Asn Phe Asn Gln Tyr Val Arg Asp Gln Gly Ala Met Thr
        35                  40                  45

Asp Gln Leu Ser Arg Arg Gln Ile Arg Glu Tyr Gln Leu Tyr Ser Arg
    50                  55                  60

Thr Ser Gly Lys His Val Gln Val Thr Gly Arg Arg Ile Ser Ala Thr
65                  70                  75                  80

Ala Glu Asp Gly Asn Lys Phe Ala Lys Leu Ile Val Glu Thr Asp Thr
                85                  90                  95

Phe Gly Ser Arg Val Arg Ile Lys Gly Ala Glu Ser Glu Lys Tyr Ile
            100                 105                 110

Cys Met Asn Lys Arg Gly Lys Leu Ile Gly Lys Pro Ser Gly Lys Ser
        115                 120                 125

Lys Asp Cys Val Phe Thr Glu Ile Val Leu Glu Asn Asn Tyr Thr Ala
    130                 135                 140

Phe Gln Asn Ala Arg His Glu Gly Trp Phe Met Ala Phe Thr Arg Gln
145                 150                 155                 160

Gly Arg Pro Arg Gln Ala Ser Arg Ser Arg Gln Asn Gln Arg Glu Ala
                165                 170                 175

His Phe Ile Lys Arg Leu Tyr Gln Gly Gln Leu Pro Phe Pro Asn His
            180                 185                 190

Ala Glu Lys Gln Lys Gln Phe Glu Phe Val Gly Ser Ala Pro Thr Arg
        195                 200                 205

Arg Thr Lys Arg Thr Arg Arg Pro Gln Pro Leu Thr
    210                 215                 220

<210> 21
<211> 207
<212> PRT
<213> Homo sapiens

<400> 21
Met Tyr Ser Ala Pro Ser Ala Cys Thr Cys Leu Cys Leu His Phe Leu
  1               5                  10                  15

Leu Leu Cys Phe Gln Val Gln Val Leu Val Ala Glu Glu Asn Val Asp
            20                  25                  30

Phe Arg Ile His Val Glu Asn Gln Thr Arg Ala Arg Asp Asp Val Ser
        35                  40                  45

Arg Lys Gln Leu Arg Leu Tyr Gln Leu Tyr Ser Arg Thr Ser Gly Lys
    50                  55                  60

His Ile Gln Val Leu Gly Arg Arg Ile Ser Ala Arg Gly Glu Asp Gly
65                  70                  75                  80
```

Asp Lys Tyr Ala Gln Leu Leu Val Glu Thr Asp Thr Phe Gly Ser Gln
85 90 95

Val Arg Ile Lys Gly Lys Glu Thr Glu Phe Tyr Leu Cys Met Asn Arg
100 105 110

Lys Gly Lys Leu Val Gly Lys Pro Asp Gly Thr Ser Lys Glu Cys Val
115 120 125

Phe Ile Glu Lys Val Leu Glu Asn Asn Tyr Thr Ala Leu Met Ser Ala
130 135 140

Lys Tyr Ser Gly Trp Tyr Val Gly Phe Thr Lys Lys Gly Arg Pro Arg
145 150 155 160

Lys Gly Pro Lys Thr Arg Glu Asn Gln Gln Asp Val His Phe Met Lys
165 170 175

Arg Tyr Pro Lys Gly Gln Pro Glu Leu Gln Lys Pro Phe Lys Tyr Thr
180 185 190

Thr Val Thr Lys Arg Ser Arg Arg Ile Arg Pro Thr His Pro Ala
195 200 205

<210> 22
<211> 216
<212> PRT
<213> Homo sapiens

<300>
<303> Biochim. Biophys. Acta
<304> 1444
<306> 148-151
<307> 1999

<400> 22
Met Arg Ser Gly Cys Val Val Val His Val Trp Ile Leu Ala Gly Leu
1 5 10 15

Trp Leu Ala Val Ala Gly Arg Pro Leu Ala Phe Ser Asp Ala Gly Pro
20 25 30

His Val His Tyr Gly Trp Gly Asp Pro Ile Arg Leu Arg His Leu Tyr
35 40 45

Thr Ser Gly Pro His Gly Leu Ser Ser Cys Phe Leu Arg Ile Arg Ala
50 55 60

Asp Gly Val Val Asp Cys Ala Arg Gly Gln Ser Ala His Ser Leu Leu
65 70 75 80

Glu Ile Lys Ala Val Ala Leu Arg Thr Val Ala Ile Lys Gly Val His
85 90 95

Ser Val Arg Tyr Leu Cys Met Gly Ala Asp Gly Lys Met Gln Gly Leu
100 105 110

Leu Gln Tyr Ser Glu Glu Asp Cys Ala Phe Glu Glu Glu Ile Arg Pro
115 120 125

```
Asp Gly Tyr Asn Val Tyr Arg Ser Glu Lys His Arg Leu Pro Val Ser
    130                 135                 140

Leu Ser Ser Ala Lys Gln Arg Gln Leu Tyr Lys Asn Arg Gly Phe Leu
145                 150                 155                 160

Pro Leu Ser His Phe Leu Pro Met Leu Pro Met Val Pro Glu Glu Pro
                165                 170                 175

Glu Asp Leu Arg Gly His Leu Glu Ser Asp Met Phe Ser Ser Pro Leu
            180                 185                 190

Glu Thr Asp Ser Met Asp Pro Phe Gly Leu Val Thr Gly Leu Glu Ala
        195                 200                 205

Val Arg Ser Pro Ser Phe Glu Lys
    210                 215
```

<210> 23
<211> 211
<212> PRT
<213> Homo sapiens

<400> 23

```
Met Ala Pro Leu Ala Glu Val Gly Gly Phe Leu Gly Gly Leu Glu Gly
  1               5                  10                  15

Leu Gly Gln Gln Val Gly Ser His Phe Leu Leu Pro Pro Ala Gly Glu
            20                  25                  30

Arg Pro Pro Leu Leu Gly Glu Arg Arg Ser Ala Ala Glu Arg Ser Ala
        35                  40                  45

Arg Gly Gly Pro Gly Ala Ala Gln Leu Ala His Leu His Gly Ile Leu
    50                  55                  60

Arg Arg Arg Gln Leu Tyr Cys Arg Thr Gly Phe His Leu Gln Ile Leu
65                  70                  75                  80

Pro Asp Gly Ser Val Gln Gly Thr Arg Gln Asp His Ser Leu Phe Gly
                85                  90                  95

Ile Leu Glu Phe Ile Ser Val Ala Val Gly Leu Val Ser Ile Arg Gly
            100                 105                 110

Val Asp Ser Gly Leu Tyr Leu Gly Met Asn Asp Lys Gly Glu Leu Tyr
        115                 120                 125

Gly Ser Glu Lys Leu Thr Ser Glu Cys Ile Phe Arg Glu Gln Phe Glu
    130                 135                 140

Glu Asn Trp Tyr Asn Thr Tyr Ser Ser Asn Ile Tyr Lys His Gly Asp
145                 150                 155                 160

Thr Gly Arg Arg Tyr Phe Val Ala Leu Asn Lys Asp Gly Thr Pro Arg
                165                 170                 175

Asp Gly Ala Arg Ser Lys Arg His Gln Lys Phe Thr His Phe Leu Pro
```

180 185 190

Arg Pro Val Asp Pro Glu Arg Val Pro Glu Leu Tyr Lys Asp Leu Leu
195 200 205

Met Tyr Thr
210

<210> 24
<211> 209
<212> PRT
<213> Homo sapiens

<300>
<303> Biochim. Biophys. Acta
<307> 2000

<400> 24
Met Asp Ser Asp Glu Thr Gly Phe Glu His Ser Gly Leu Trp Val Ser
1 5 10 15

Val Leu Ala Gly Leu Leu Leu Gly Ala Cys Gln Ala His Pro Ile Pro
20 25 30

Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr
35 40 45

Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg
50 55 60

Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu
65 70 75 80

Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val
85 90 95

Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly
100 105 110

Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu
115 120 125

Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu
130 135 140

His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly
145 150 155 160

Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu Pro Glu
165 170 175

Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp
180 185 190

Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Tyr Ala
195 200 205

Ser

<210> 25
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR primer

<400> 25
cgacgagcgc gcagcgaac 19

<210> 26
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR primer

<400> 26
ctctcagggc ctcaggaga 19

<210> 27
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR primer

<400> 27
aaccgggtgc cgggtcatg 19

<210> 28
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR primer

<400> 28
gcctcaggag accaggac 18

<210> 29
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:protein tag

<400> 29
Gly Ala Pro Val Pro Tyr Pro Asp Pro Leu Glu Pro Arg
1 5 10

<210> 30
<211> 6
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:protein tag

<400> 30
His His His His His His
1 5

<210> 31
<211> 489
<212> DNA
<213> Mus sp.

<400> 31
atgcgcagcc gcctctggct gggcctagcc tggctgctgt tggcgcgggc accgggcgct 60
ccgggagggt acccgcatct ggagggcgac gtgcgctggc gccgcctctt ctcctccact 120
cacttttcc tgcgtgtgga ccttggtggt cgggtgcagg ggacgcgttg gcggcacggc 180
caggacagta tagtggagat ccgttctgtc cgtgtgggca ctgtggtgat caaagctgtg 240
tactcaggct tctatgtggc catgaatcgc aggggccgcc tctatgggtc gcgggtctac 300
tctgtggact gtaggttccg ggagcgcatc gaggagaacg gctacaacac atacgcctcg 360
cgacgttgga ggcaccgcgg ccgacccatg ttcctggcac ttgacagcca aggcattccc 420
aggcaaggca gacggacacg acggcaccaa ctgtccacac acttcctgcc agtcttggtc 480
tcgtcttga 489

<210> 32
<211> 162
<212> PRT
<213> Mus sp.

<400> 32
Met Arg Ser Arg Leu Trp Leu Gly Leu Ala Trp Leu Leu Leu Ala Arg
1 5 10 15

Ala Pro Gly Ala Pro Gly Gly Tyr Pro His Leu Glu Gly Asp Val Arg
20 25 30

Trp Arg Arg Leu Phe Ser Ser Thr His Phe Phe Leu Arg Val Asp Leu
35 40 45

Gly Gly Arg Val Gln Gly Thr Arg Trp Arg His Gly Gln Asp Ser Ile
50 55 60

Val Glu Ile Arg Ser Val Arg Val Gly Thr Val Val Ile Lys Ala Val
65 70 75 80

Tyr Ser Gly Phe Tyr Val Ala Met His Arg Arg Gly Arg Leu Tyr Gly
85 90 95

Ser Arg Val Tyr Ser Val Asp Cys Arg Phe Arg Glu Arg Ile Glu Glu
100 105 110

Asn Gly Tyr Asn Thr Tyr Ala Ser Arg Arg Trp Arg His Arg Gly Arg
115 120 125

Pro Met Phe Leu Ala Leu Asp Ser Gln Gly Ile Pro Arg Gln Gly Arg
130 135 140

Arg Thr Arg Arg His Gln Leu Ser Thr His Phe Leu Pro Val Leu Val

145 150 155 160

Ser Ser

<210> 33
<211> 140
<212> PRT
<213> Mus sp.

<400> 33
Gly Tyr Pro His Leu Glu Gly Asp Val Arg Trp Arg Arg Leu Phe Ser
1 5 10 15

Ser Thr His Phe Phe Leu Arg Val Asp Leu Gly Gly Arg Val Gln Gly
20 25 30

Thr Arg Trp Arg His Gly Gln Asp Ser Ile Val Glu Ile Arg Ser Val
35 40 45

Arg Val Gly Thr Val Val Ile Lys Ala Val Tyr Ser Gly Phe Tyr Val
50 55 60

Ala Met His Arg Arg Gly Arg Leu Tyr Gly Ser Arg Val Tyr Ser Val
65 70 75 80

Asp Cys Arg Phe Arg Glu Arg Ile Glu Glu Asn Gly Tyr Asn Thr Tyr
85 90 95

Ala Ser Arg Arg Trp Arg His Arg Gly Arg Pro Met Phe Leu Ala Leu
100 105 110

Asp Ser Gln Gly Ile Pro Arg Gln Gly Arg Arg Thr Arg Arg His Gln
115 120 125

Leu Ser Thr His Phe Leu Pro Val Leu Val Ser Ser
130 135 140

<210> 34
<211> 995
<212> DNA
<213> Mus sp.

<400> 34
ctggagctcg cgcgcctgca ggtcgactag tacgggggg ggggggggg accacttgtc 60
ctcagacaca tcccaccaaa cccgcacacc atcctgcaac gagacagatg acaagacgga 120
cacgacggca caggtcggga tgcgcagccg cctctggctg ggcctagcct ggctgctgtt 180
ggcgcgggca ccgggcgctc cgggagggta cccgcatctg gagggcgacg tgcgctggcg 240
ccgcctcttc tcctccactc acttttcct gcgtgtggac cttggtggtc gggtgcaggg 300
gacgcgttgg cggcacggcc aggacagtat agtggagatc cgttctgtcc gtgtgggcac 360
tgtggtgatc aaagctgtgt actcaggctt ctatgtggcc atgaatcgca ggggccgcct 420
ctatgggtcg cgggtctact ctgtggactg taggttccgg gagcgcatcg aggagaacgg 480
ctacaacaca tacgcctcgc gacgttggag gcaccgcggc cgacccatgt tcctggcact 540
tgacagccaa ggcattccca ggcaaggcag acggacacga cggcaccaac tgtccacaca 600
cttcctgcca gtcttggtct cgtcttgaag ggcctgccaa tggttcagga ggcatgaatc 660
actagtgaat tcaaaaagct tctcgagagt acttctagag cggccgcggg cccatcgatt 720
ttccacccgg gtggggtacc aggtaagtgt acccaattcg ccctatagtg agtcgtatta 780
caattcactg gccgtcgttt tacaacgtcg tgactgggaa aaccctggcg ttacccaact 840
taatcgcctt gcagcacatc ccccttcgc cagctggcgt aatagcgaag aggcccgcac 900
cgatcgccct tcccaacagt tgcgcagcct gaatggcgaa tggagatcca atttttaagt 960

gtataatgtg ttaaactact gattctaatt gtttg 995

<210> 35
<211> 162
<212> PRT
<213> Mus sp.

<400> 35
Met Arg Ser Arg Leu Trp Leu Gly Leu Ala Trp Leu Leu Leu Ala Arg
1 5 10 15

Ala Pro Gly Ala Pro Gly Gly Tyr Pro His Leu Glu Gly Asp Val Arg
20 25 30

Trp Arg Arg Leu Phe Ser Ser Thr His Phe Phe Leu Arg Val Asp Leu
35 40 45

Gly Gly Arg Val Gln Gly Thr Arg Trp Arg His Gly Gln Asp Ser Ile
50 55 60

Val Glu Ile Arg Ser Val Arg Val Gly Thr Val Val Ile Lys Ala Val
65 70 75 80

Tyr Ser Gly Phe Tyr Val Ala Met Asn Arg Arg Gly Arg Leu Tyr Gly
85 90 95

Ser Arg Val Tyr Ser Val Asp Cys Arg Phe Arg Glu Arg Ile Glu Glu
100 105 110

Asn Gly Tyr Asn Thr Tyr Ala Ser Arg Arg Trp Arg His Arg Gly Arg
115 120 125

Pro Met Phe Leu Ala Leu Asp Ser Gln Gly Ile Pro Arg Gln Gly Arg
130 135 140

Arg Thr Arg Arg His Gln Leu Ser Thr His Phe Leu Pro Val Leu Val
145 150 155 160

Ser Ser